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The Gametocyte Development of *Leucocytozoon simondi*[1]

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The type of host cell involved in the gametocyte development of species of the genus *Leucocytozoon* has been the subject of much controversy. The extensive literature, which has been reviewed by Huff (1942), reveals little agreement beyond the fact that the gametocytes are found in the circulating blood. O'Roke (1934) was of the opinion that the gametocytes of *L. simondi* developed in red blood cells of the duck, although he reported finding the earliest gametocytes in all types of leucocytes as well as in erythrocytes. Hartman (1929) described schizogony and gametogony both taking place in the duck red corpuscles. Fallis *et al* (1951) reported finding young parasites in erythrocytes and lymphocytes. Huff (1942), in a detailed histological study of gametocyte development, stated that the only cells in which he could "find closely spaced stages of growth from the smallest forms to the fully grown gametocytes were the lymphocytes and the stages in transformation between them and monocytes." The following observations on *L. simondi* are an attempt to clarify the contradictory descriptions of the peripheral blood stages of this parasite. Because of the nature of the material available, the study deals primarily with the stages associated with the round gametocytes.

Material for this study was obtained during the summers of 1951 and 1952 at the University of Michigan Biological Station. The hatchery-raised White Pekin ducks (*Anas platyrhynchos*) used as hosts, were kept in a black-fly proof animal house before and after exposure in the enzootic area. This same method was used by Chernin (1952a). All blood smears were fixed in methyl alcohol and stained with Giemsa. To detect the presence of haemoglobin in red blood cells, smears were then treated by the benzidine-peroxide method described by Ralph (1941), which did not impair the differentiating capacity of the Giemsa stain.

During the last week in July, 1951, 30 ducks, six weeks old, were placed on local farms in Emmet and Cheboygan Counties. This exposure period anticipated the height of the epizootic in the area (Chernin, 1952a). Eighteen ducks were allowed to remain on the farms for only 4 hours, another 4 ducks for 24 hours, while 2 groups of 4 ducks each remained exposed for 8 days.

Examinations of blood smears made at the onset of patency revealed the presence of small rings, similar in appearance to those found in the bird malarias, in obvious red blood cells. At first it was thought that the parasite

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might be *Haemoproteus,* but no blood parasite other than *Leucocytozoon* was found over the period of 14 days in which smears were taken. Moreover, in some 100 ducks followed during the summers of 1951-1952, *L. simondi* was the only haemosporidian seen. All but 4 of the 30 ducks exposed became blood positive for *L. simondi;* 12 succumbed to the infection and 2 others were killed when found moribund.

The detailed studies reported below were made from the birds exposed for eight days because of the ease of locating young stages of the parasite in these birds as compared with the problem of finding them in birds exposed for shorter periods of time. The 4 ducks in Group I were placed on an Emmet County farm from 7-26-51 to 8-3-51. Group II ducks were placed on a Cheboygan County farm from 7-25-51 to 8-2-51. In smears taken 8 and 9 days after the beginning of exposure, ring stages of the parasite were present along with growth stages from rings to large round gametocytes. In lightly infected birds ring stages were seen only in erythrocytes. In the more heavily infected birds rings were seen in lymphocytes as well as in red blood cells, but appeared to be more numerous in the latter. Some of the host cells which contained young trophozoites could also be identified as erythrocytes. As the trophozoites grew the host cell became more distorted until it was no longer recognizable as an erythrocyte in the material stained with Giemsa alone.

Since preliminary studies revealed that young ring stages were found in considerable numbers in both erythrocytes and lymphocytes, an attempt was made to determine whether or not both of these cells were included among the distorted and histologically unrecognizable cells containing the later stages. Specific histochemical staining of lymphocytes was attempted unsuccessfully. Accordingly, the significant results came only from the use of a specific stain for haemoglobin. With this technic parasitized cells in various stages of distortion could be identified as erythrocytes and a developmental series pieced together.

Haemoglobin stains a golden-brown with the benzidine-peroxide method. The cytoplasm of a normal mature red cell stains uniformly this color (Fig. 1). Reticulocyte cytoplasm stains one of two ways, either unevenly with the haemoglobin in clumps (Fig. 2) or homogeneously golden-brown with a bluish-gray cast of the Giemsa counterstain; the nucleus is larger and not as compact as that of a mature erythrocyte. Judging by the staining quality of the cytoplasm and the degree of enlargement of the nucleus, it appeared that about 2/3 of the rings in the erythrocyte-series were actually in reticulocytes. The use of a specific stain for reticulum might reveal an even greater preference for young red blood cells. Cells infected with small rings appear the same as their non-infected counterparts. As the parasite increases in size, the host cell enlarges and becomes more or less rounded; the nucleus enlarges, loses its oval contour and usually assumes a kidney-bean shape with the parasite nestled in the indentation (Figs. 3, 4, 5 & 6). During this period in the growth of the parasite, there is a gradual diminuation in the haemoglobin content of the host cell cytoplasm. In such cells the cytoplasm either stains uniformly light brown or is clumped into a few light-brown staining aggregates. In cells which have reached the size of 20 μ it is still possible to observe some brown cytoplasm (Fig. 8). The nucleus of the host cell becomes more vacuolated and elongated as the parasite grows, until it is no more than a ribbon of dark purple, circling half-way around or along one side of the parasite (Figs. 7 & 8). Micro- and macrogametocytes are distinguishable

Blood cells from smear of Duck #85 taken 9 days after beginning of exposure to natural infection with *L. simondi.* Slide was stained with Giemsa and then treated with benzidine and peroxide. Intensity of stippling in cytoplasm corresponds to the varying amounts of golden-brown haemoglobin stain in the cytoplasm of the host cell.

Fig. 1. Mature erythrocyte with haemoglobin uniformly distributed.

Fig. 2. Reticulocyte; haemoglobin less evenly distributed and containing ring stage of *L. simondi.*

Figs. 3 thru 8. Progressive stages in the growth of the gametocytes showing gradual diminuation in the haemoglobin content of the host cell cytoplasm. Figs. 7 and 8 are respectively the young microgametocyte and macrogametocyte, which develop into the mature round gametocytes.

Fig. 9. Lymphocyte containing 3 rings. Red chromatin dots are clearly evident. Cytoplasm of parasite and host cell are not clearly distinguishable from each other.

before the parasite completely fills the host cell. The nucleus of the microgametocyte is diffuse and stains a light pink (Fig. 7), while that of the macrogametocyte is concentrated and contains a nucleolus that stains deep red (Fig. 8). With the combined Giemsa and benzidine-peroxide stain, lymphocytes and other white cells show no change in appearance from an ordinary Giemsa stain. The nucleus is a crimson-purple and the cytoplasm stains distinctly blue. Rings were found in no white cells other than lymphocytes. Multiple infection was common with as many as 5 or 6 parasites contained in one lymphocyte (Fig. 9).

Counts were made of the various growth stages of the parasite found in different host cells. Where the parasites were plentiful, 100 were counted and where they were less abundant as many as could be found without prolonged search were counted. Since these smears were taken during early patency, it will be noted that 25% of the total number of parasites counted were small rings (Table I). Of these, 14% were in cells whose cytoplasm contained haemoglobin and were easily identified as erythrocytes before histochemical staining was attempted. The other 11% were found in lymphocytes. Group II ducks evidenced a higher parasitemia than the members of Group I and it is probably for this reason that so many rings found in lymphocytes were accountable to this group. Many of these lymphocytes were found to be multiply infected; a factor which contributed to elevating the count. Only the chromatin dot of the ring within the lymphocyte can be clearly differentiated (Fig. 9), since the cytoplasm of both the lymphocyte and the parasite stains blue. Of the total parasites counted, 42% were growth stages of gametocytes (Figs. 3 thru 8) in cells showing some evidence of haemoglobin. No developing stages were found in lymphocytes or other members of the white cell series. The parasite continues to grow until it completely fills the host cell. In the usual Giemsa stain the host cell membrane can sometimes be distinguished by carefully adjusting the light and the cytoplasm of the cell is either unstained or stains a very faint blue. At this stage of development it was difficult to determine whether or not the cytoplasm showed any brown stain.

TABLE I.—Numbers of parasites found in blood cells of ducks exposed to natural infection for eight days.

			Ring stages		Developing stages of gametocytes		Large round gametocytes
	Duck no.	Total no. parasites counted	In cells showing positive test for haemoglobin	In lympho-cytes	In cells showing positive test for Hb.	In lympho-cytes	In cells without clear positive test for haemoglobin
GROUP I	91	50	3	0	5	0	42
	78	25	0	0	4	0	21
8 days after beginning of exposure	77	25	0	0	1	0	24
	76	2	1	0	1	0	0
GROUP II	85	100	22	27	30	0	21
	72	100	19	11	65	0	5
9 days after beginning of exposure	75	100	13	8	64	0	15
	100	50	3	3	21	0	23
TOTALS		452	61	49	191	0	151
		100	14	11	42	0	33

In some cases a faint tinge of golden-brown seemed detectable but where there was any question of a clear positive test for haemoglobin, the cell was relegated to the haemoglobin-negative group. Therefore 33% of the total number of parasites counted were large round gametocytes in cells of unknown classification.

No elongate gametocytes were present at this stage in the infection. In slides taken from older infections after the elongate forms had appeared, it was not possible to demonstrate haemoglobin in the fusiform host cell. This was to be expected since in Giemsa stained material it is difficult to see anything other than the host cell wall. The area between the host cell wall and the parasite is usually colorless or stains extremely faint grey. Although it is usually assumed that the elongate stage is the terminal gametocyte form in the duck, no transitional stages were seen from round to elongate gametocytes. In one heavily parasitized duck, parasites were seen which could be interpreted as young stages in the development of the elongate gametocytes. In smears from this duck taken 14 days after infection both typical large round and large elongate gametocytes were seen in host cells which were so distorted that their identity could not be determined. In addition, there were parasite gradients from rings and young trophozoites through young gametocytes to the largest round gametocytes. A few young gametocytes were seen in slightly elongate cells which suggested possible young stages in the development of elongate gametocytes. These appeared to be in red blood cells identical in appearance with the host cell containing stages of round gametocytes, but a complete series of growth stages could not be traced. No forms were seen which indicated a transition between the largest round gametocytes and the large elongate forms.

Chernin (1952b) found that the round gametocytes reached their maximum concentration on the fourth day of patency while the elongate ones reached a peak on the ninth day of patency by which time the round ones had nearly disappeared. Fallis et al (1951) transfused blood containing both round and elongate gametocytes into clean ducks and found both types present several days thereafter. In the course of the studies reported here, opportunity was afforded to do one transfusion experiment which bears on this subject. Thirteen cubic centimeters of heparinized blood, in which only round gametocytes were found was drawn by cardiac puncture from a duck (#506) on the 2nd day of patency, 10th day after infection, and inoculated into the leg vein of a clean bird (#581). Round gametocytes were found in the blood of the recipient duck for seven days. Extensive search of smears taken up to 11 days after the transfusion failed to reveal any elongate gametocytes.

Since the presence or absence of pigment in the gametocytes of the various species of *Leucocytozoon* has been open to question, particular attention was paid to all small bodies contained in the cytoplasm of the parasite that might resemble malaria pigment. On numerous occasions dark purple dots, apparently the "pseudopigment" of Wingstrand (1947), were seen in the cytoplasm of those round parasites which appeared to have attained full growth. There was a marked variation in the size of these granules, which were evenly distributed throughout the cytoplasm (Fig. 8). The same cytological structures were in the elongate gametocytes of birds with older infections. These granules were easily made out in certain areas of the giemsa-stained slides, while in other parts of the same slide they could not be distinguished at all. Some of these slides showing granules in gametocytes were treated with

acidified water to remove the stain. After this treatment the granules were no longer discernible. Slides of *Plasmodium gallinaceum* treated in the same manner showed no change in the appearance or amount of pigment granules. Wingstrand (1947) treated slides of *Leucocytozoon* spp. with acetic acid and ammonia, after which the granules disappeared, whereas the pigment of *Haemoproteus* remained after similar treatment. The granules do not resemble malaria pigment in any of their optical qualities and no granules optically similar to malaria pigment were seen in any of the stages of *L. simondi* studied.

DISCUSSION

One of the earliest and most easily recognized symptoms in birds infected with *L. simondi* is anemia. Therefore, it is not surprising to find that the parasite develops in red cells rather than leucocytes. Fallis *et al* (1951) reported from detailed haematological studies on infected ducks that numbers of red cells, blood cell volume and the amount of haemoglobin decreased as the infection appeared in the blood, with a corresponding increase in the total leucocyte count. This pattern of blood change fits with the evidence that the parasite invades primarily red cells and consequently in heavy infections destroys great numbers of them.

The problems arising in the classification of the host cells which are invaded by various species of *Leucocytozoon* have already been indicated by Huff (1942). He concluded that the severe distortion of the host cell was responsible for the difficulties in identification, Since it is impossible to recognize the host cell containing advanced growth stages of gametocytes the only time an identification can be made is before the parasite has caused any severe change in the original morphology of the cell. This suggests that if any work is to be done on the identity of the host cell of the leucocytozoa of other birds, it will be necessary to study the youngest stages seen in the blood. Single smears from birds during the course of a blood parasite survey do not constitute adequate material for study, unless one is fortunate enough to obtain the smears at the beginning of patency in heavily infected birds when all growth stages of the parasite are present.

Huff (1942) states that the parasitized cells may be recognized definitely as lymphocytes or monocytes some time during the development of the gametocyte. It should be noted here that as the haemoglobin content of the parasitized red cell diminishes, the cytoplasm stains blue with the usual Romanowsky stains. The nucleus of the red cell greatly enlarges and does not stain as densely as does the nucleus of a normal mature erythrocyte. These changes result in the over-all similarity of the parasitized cell to a large lymphocyte. Huff (1932) in discussing a species of *Haemoproteus* that was confused with a *Leucocytozoon,* states that the fully grown gametocyte so enlarged and distorted the host cell, that it was necessary to study the young stages to determine that the host cell of the *Haemoproteus* was an erythrocyte. Such appears to be the case with *L. simondi.*

Huff (1942) observed the smallest stages of *L. simondi* in late "polychromatophil erythroblasts" and lymphocytes, but growth stages only in lymphocytes and monocytes. No ring forms are shown in his illustrations and since apparently wet-fixed smears and sections were used as a basis for determining his developmental series, it is difficult to compare his material with that studied in the present work. Sectioned material is not satisfactory for study-

ing the morphology of blood cells since it is difficult to get the azur-eosin contrast for nucleus and cytoplasm. Apparently no differentiation by histochemical staining was attempted.

The account by Hartman (1929) of the growth of *L. simondi* from rings to large round forms, agrees in part with gametocyte development reported in this paper. However, he describes one stage in gametocyte growth in which the host cell nucleus breaks up and the protoplasm of the parasite surrounds it. No such stage was observed during the course of this investigation and it is possible that he was dealing with the lymphocytes multiply infected by rings which are seen in the more heavily infected birds. No organisms were observed which would support the inference of Hartman that schizogony occurs, however rare, in the peripheral blood or that the round forms develop into the elongate gametocytes. O'Roke (1934) was also of the opinion that the elongate gametocytes were the "mature" forms and developed from the round ones which he considered were "nearly mature." Neither of the above mentioned authors gave descriptions of the forms intermediate between these two. No evidence of growth stages between round and elongate gametocytes were found in any of the birds used for this study and no stages which could be interpreted as schizonts were seen.

Since transitional stages between the two forms of gametocytes could not be demonstrated, it was interesting to find that upon transfusion of round forms, when they were the sole peripheral blood stage in evidence, there was no development beyond that stage in the blood of the recipient bird. This supports the findings of Chernin (1952b), who was unable to correlate the densities of round gametocyte populations with the subsequent number of elongate gametocytes. Reference was also made by the same author to 4 birds in which round gametocytes were the only parasites observed over a period of a month. According to Fallis et al (1951) both round and elongate gametocytes of *L. simondi* exflagellate. This phenomenon was also observed by Rawley (1953) and seen by the author. In the light of these findings, it is difficult to accept the terminology of "immature" and "mature" for the two types of gametocytes, but rather to consider both forms as being mature.

There is no other known haemosporidian group which possesses two morphologically different gametocytes, therefore, it is necessary to consider other explanations for these observations in *L. simondi.* Fallis et al (1951) suggested that two types of host cells might be involved, one of which might elongate soon after the parasite enters. This explanation is a decided possibility but there are some features of the disease and of the life cycle of the parasite that do not fit. The eight birds in Groups I and II all showed round gametocytes in the peripheral blood before they showed the elongate type. This pattern of gametocytemia is the same as that reported by Chernin (1952b). Therefore, if the shape of the host cell and the gametocyte contained within it is dependent solely upon the types of host cell invaded by the parasite, then both round and elongate gametocytes should appear simultaneously in the peripheral blood, unless the host cell of the elongate gametocyte exerts an inhibitory affect on the growth of the parasite. In addition, young parasites should be observed in the two types of blood cells, but the present author was unable to find young trophozoites in any cells other than red blood cells throughout the peripheral blood parasitemia. It was possible to trace a complete developmental series for the round gametocytes in the peripheral blood. However, a similar series could not be established for the

elongate gametocytes, although certain stages were found suggestive of young elongate forms and these also appeared to be in red blood cells. Perhaps the physiological differences between reticulocytes and mature erythrocytes could lead to a different morphological appearance of the parasites which infect them. However, since reticulocytes seem to be the primary host cell for the round gametocytes which appear first, it is hardly likely that the later occurrence of elongate gametocytes would develop in erythrocytes when the severe anemia accompanying the disease decreases markedly the number of mature red blood cells present in the blood. It is evident that more work is needed on this subject before any explanation can be accepted or rejected completely. Accordingly, it seems appropriate to consider other possible explanations upon which future work can be based.

There are species of leucocytozoa reported that appear to have only one type of gametocyte. Recently, Atchley (1951) erected a new species, *L. andrewsi*, for a form found in chickens where only round gametocytes were demonstrable, since in previously described species from chickens, both round and elongate forms were found. Manwell (1951) found only round gametocytes occurring in the purple grackle. Both of the above reports were based on the examinations of a considerable number of slides and had two forms actually existed, it seems likely that they would have been observed. Therefore, considering gametocyte morphology as a species characteristic, the two morphologically different gametocytes could be accounted for if one took into account the possibility of the simultaneous transmission of two different species of *Leucocytozoon*. There are no reports of species of *Leucocytozoon* in which only elongate gametocytes have been observed throughout the full length of the primary parasitemia. It will be recalled that Chernin (1952b) observed only four ducks which showed round gametocytes of *L. simondi* alone for a period of a month, but in no bird were the elongate gametocytes the sole evidence of infection. If two species of *Leucocytozoon* are being transmitted simultaneously, either by the same insect vector or different ones with similar ecology, one would expect to find many more birds harboring only one species of parasite. Huff (1930), working with bird malaria and *Culex pipiens* found extreme variation in the infectivity of the vector with different species of malaria. Not all of the mosquitos given an opportunity to become infected with two species of malaria did so. Some became infected with only one of the two while others, of course, remained uninfected. It would be unique in the haemosporidians if two species of *Leucocytozoon* were being transmitted simultaneously so regularly.

Huff (1942) described two types of schizonts in *L. simondi* infection, a small schizont in the liver, which he termed "hepatic schizont" as opposed to the "megaloschizont," a larger form which he found in the spleen, heart and intestine. Fallis et al (1951) were only able to find the megaloschizonts. If two types of schizogonic forms do exist, possibly the two forms of gametocytes could be derived from the two different schizonts. However, a type of development which has two sets of sexual elements is unknown to the Sporozoa and a truly analogous development is unknown in the protozoa as a whole.

Since none of the proposed explanations can be reconciled with the known evidence of the life cycle of this parasite, much remains to be done before the true nature of the round and elongate gametocytes can be elucidated, but it does appear evident that at least the round gametocytes develop in cells of the

erythrocytic series and a limited number of observations indicate the same is true for the elongate gametocytes. Both round and elongate gametocytes appear to be mature and neither contain pigment comparable to that seen in *Plasmodium* and *Haemoproteus*.

SUMMARY

Evidence is presented that the gametocytes of *L. simondi* found in the circulating blood of the duck, develop exclusively in the red blood cell series. Both the round and elongate gametocytes appear to be mature and no transitional stages between the two could be found. No evidence of pigment was seen in either form.

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Studies on Bovine Gastro-Intestinal Parasites XVII. Feeding small amounts of phenothiazine during the prepatent period in pure infections of the nodular worm.

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In previous publications of this series, I have reported the results of feeding small amounts of phenothiazine in pure infections of the hookworm (Mayhew 1950) and of the nodular worm (Mayhew 1951 and 1952) during the adult life of the parasites. In the following pages certain experiments are described in which the drug was fed during the prepatent period of the nodular worm.

RESULTS WHEN 1.5 GRAM WERE FED DURING THE FIRST TWENTY-EIGHT DAYS OF THE PREPATENT PERIOD. Calf No. 219 (Fig. 1), a Jersey-Guernsey crossbred male, born on October 25, 1950, was inoculated with 25,000 larvae on April 14, 1951. Beginning twelve days before inoculation and continuing until 28 days after inoculation, 1.5 grams of phenothiazine were fed daily (April 2 to May 12). It will be noted (Fig. 1) that no eggs were recovered from the feces up to the fifty-third day after inoculation. The calf thus remained negative at least ten days beyond the maximum time when eggs usually are first recovered and the resulting counts were very low.

Calves Nos. 221 and 222 (Fig. 1), grade Holstein males born on November 26, 1950, were each given 40,000 nodular worm larvae on March 28, 1951. Beginning on the preceding day, 1.5 grams of phenothiazine were fed through April 25th, 28 days after inoculation. Both animals were still negative on June 6th (Fig. 1), 70 days after inoculation and 27 days after the maximum range of the prepatent period. A few eggs were recovered from both calves on June 25th, 90 days after inoculation (Fig. 1). It was necessary to discontinue observations on No. 222, but it was possible to keep No. 221 under conditions of freedom from reinfection for three additional months. During the third month, the number of eggs recovered from No. 221 reached sufficient numbers that larvae were cultured from the feces to inoculate additional calves. This indicated that such calves may produce enough larvae to be a source of infection, consequently, continuous feeding of the drug is recommended.

Calf No. 224 (Fig. 1), a grade Holstein male born on January 8, 1951, was inoculated with two lots of larvae, 36,000 on May 11th and 15,180 on May 17, 1951. Phenothiazine was fed at the rate of 1.5 grams per day beginning 17 days before the first inoculation and continuing until 28 days after inoculation. Nine fecal examinations made between June 1 and July 10 were all negative (Fig. 1).

RESULTS OF FEEDING PHENOTHIAZINE DURING THE SECOND TWO WEEKS OF PREPATENT PERIOD. Calves Nos. 211, 212, 213 (Fig. 3) were pure bred males born between April 25th and May 15, 1950. No 211 was a Jersey and 212 and 213 were Holsteins. All were given larvae from the same culture on September 1, 1950 (No. 211, 2,000; 212, 3,600; 213, 6,000). Nos. 212 and 213 were given 1.5 grams of phenothiazine for fourteen days, beginning on the 14th day after inoculation, and No. 211 was used as a control. It will be noted (Fig. 3) that eggs were recovered from the feces of No. 211, the control animal, 32 days (October 3rd) after inoculation and that he developed a rea-

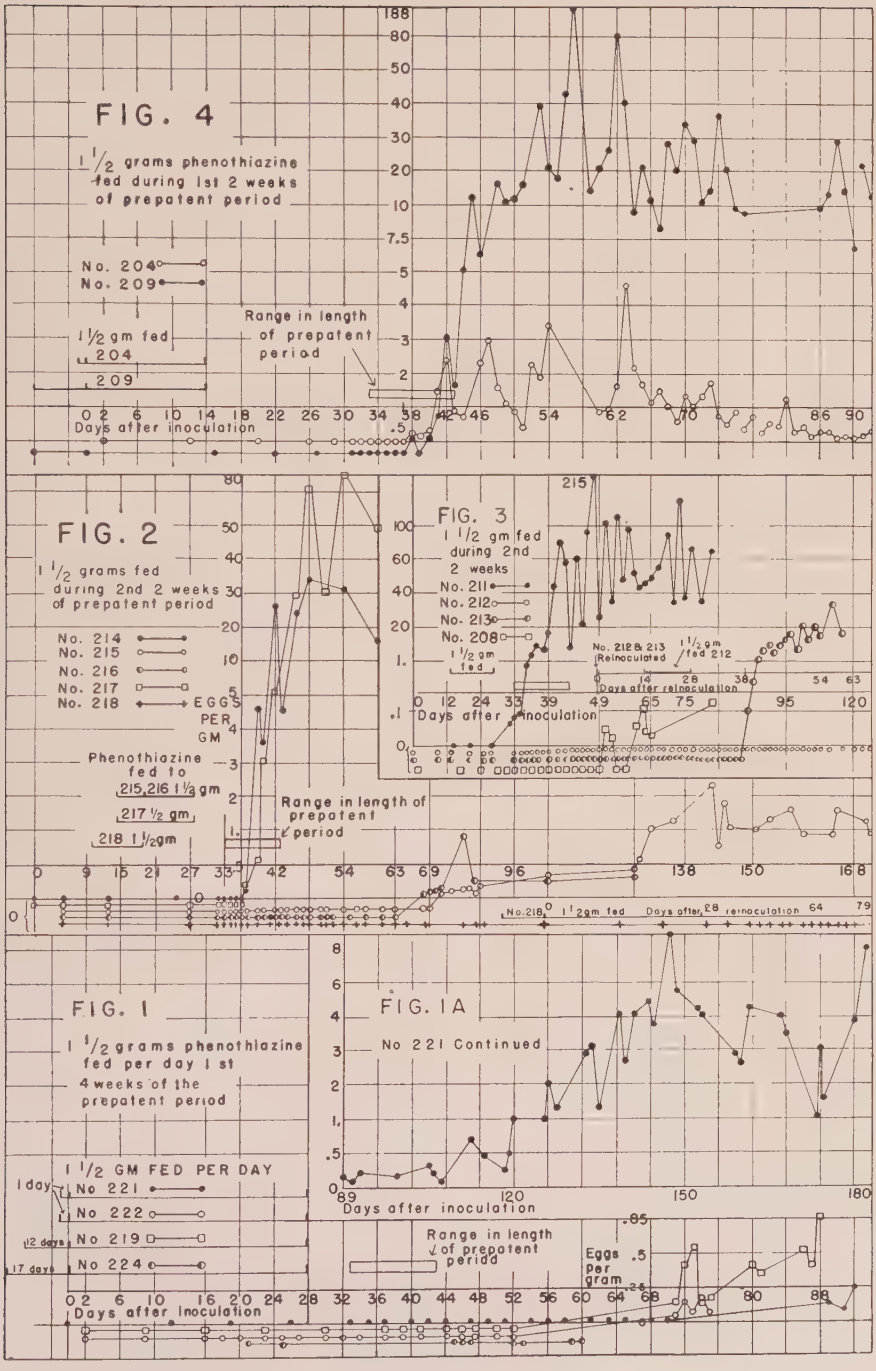
FIG. 4
1 1/2 grams phenothiazine fed during 1st 2 weeks of prepatent period
No. 204
No. 209
1 1/2 gm fed
204
209
Range in length of prepatent period
Days after inoculation
FIG. 2
1 1/2 grams fed during 2nd 2 weeks of prepatent period
No. 214
No. 215
No. 216
No. 217
No. 218
EGGS PER GM
Phenothiazine fed to
215,216 1 1/2 gm
217 1/2 gm
218 1 1/2 gm
Range in length of prepatent period
FIG. 3
1 1/2 gm fed during 2nd 2 weeks
No. 211
No. 212
No. 213
No. 208
1 1/2 gm fed
No. 218 & 213 Reinoculated
1 1/2 gm fed 212
Days after reinoculation
Days after inoculation
No. 218 1 1/2 gm fed
Days after reinoculation
FIG. 1
1 1/2 grams phenothiazine fed per day 1st 4 weeks of the prepatent period
1 1/2 GM FED PER DAY
1 day
No 221
No 222
12 days
No 219
17 days
No 224
Days after inoculation
FIG. 1A
No 221 Continued
Days after inoculation
Range in length of prepatent period
Eggs per gram
Days after inoculation

sonably high egg count, while Nos. 212 and 213 remained negative. On October 20th, Nos. 212 and 213 were reinoculated (2,000) and No. 212 was fed 1.5 grams of phenothiazine for two weeks beginning November 3rd, 14 days after reinoculation. It will be noted (Fig. 3) that eggs began to appear in the feces of No. 213, 38 days (November 27) after inoculation, while No. 212, the animal that received the drug, continued to remain negative. No. 212 remained negative for 123 days after the first inoculation and 63 days after the second as demonstrated by a total of 76 fecal examinations made between August 15th and December 22, 1950.

Calf No. 208 (Fig. 3), a pure bred Holstein male, was inoculated with 41,700 nodular worm larvae on May 10th from the same culture as was No. 209, to be described later in this paper. One and a half grams of phenothiazine was fed per day from the 14th to the 28th day after inoculation. He remained negative until 53 days (July 2nd) after inoculation (Fig. 3). Thus, the appearance of eggs in the feces was delayed 10 days and the egg count record (Fig. 3) shows that the number of eggs remained very low. No. 209 which received larvae from the same culture and as described later, developed a reasonably high egg count, indicating that the larvae that No. 208 received, were infective.

Calves Nos. 214, 215, 216, 217 and 218 (Fig. 2) were all pure bred males born between October 9th and 20, 1950. All were Jerseys except No. 216 which was a Holstein. Each of the five animals was inoculated with an estimated 5,000 nodular worm larvae from the same culture on January 13, 1951. No. 214 was not given any phenothiazine, but was used as a control on the viability of the larvae and the normal course of the infection, and it will be noted (Fig. 2) that egg production began well within the normal course of the prepatent period and a reasonably high egg count was obtained. Nos. 215 and 216 were each given 1.5 grams of the drug daily (Fig. 2) from the 15th to the 28th day after inoculation. No. 216 remained negative until 63 days after inoculation (Fig. 2) and No. 215 remained negative until 69 days after inoculation or 20 and 26 days respectively, after the end of the normal prepatent period. The egg counts of both of these animals remained low during the next three months (Fig. 2).

No. 218 (Fig. 2) received 1.5 grams of phenothiazine from the 10th to the 19th day after inoculation. All fecal examinations made on this animal were consistently negative (Fig. 2). He was reinoculated on April 9th (9.800) (Fig. 2) and was fed 1.5 grams of phenothiazine beginning April 2nd and continued till May 7th, 28 days after reinoculation. A total of 54 fecal examinations were made on this animal and all were negative. The last fecal examination was made 168 days after the first inoculation and 79 days after the last inoculation.

No. 217 was given 0.5 gram of phenothiazine daily from the 15th to the 28th day after inoculation. The resulting fecal examinations (Fig. 2) indicate that 0.5 gram is not sufficient to interfere with the normal development and egg production of the nodular worm, since a reasonably large number of eggs were recovered from the feces and egg production began on the thirty-fifth day after inoculation, at a time well within the normal limits of the prepatent period.

Results when 1.5 grams were fed during the first two weeks of the prepatent period. Calf. No. 204 (Fig. 4), a Brahman-Guernsey cross-bred female born November 11, 1949, was inoculated with 5,000 larvae on February 2, 1950. One and a half grams of phenothiazine were fed daily from

seven days before inoculation until 14 days after inoculation. The first eggs were recovered from the feces 38 days after inoculation. They appeared normal and the resulting egg counts (Fig. 4) showed that a reasonably good infection resulted from the inoculation.

Calf No. 209 (Fig. 4) a pure bred Holstein male born January 1, 1950, was inoculated with 48,180 nodular worm larvae on May 11, 1950. The feeding of 1.5 grams of phenothiazine was begun the day before inoculation, and continued for the next 14 days. The first nodular worm eggs were recovered 38 days after inoculation (Fig. 4). This is well within the normal range (33-43 days) of the prepatent period. The resulting egg counts (Fig. 4) show a reasonably large number of eggs.

The results of the experiments on the last two calves, No. 204 and 209, indicate that the normal cycle of development of the larvae had not been influenced by the phenothiazine when fed during the first two weeks of the prepatent period. Since the results of the above two experiments (Nos. 204 and 209) in which small numbers of larvae were used, indicate that there is no detrimental effect of phenothiazine on the developing larvae during the first two weeks, it was decided to inoculate with large numbers of larvae to determine if the drug would prevent symptoms. Experiments designed for this purpose are next described.

Calves Nos. 228, 229 and 233 were all pure bred Holstein males born between August 11 and 17, 1951. No. 228 was inoculated on September 29, 1951 with 133,801 nodular worm larvae. One and one-half grams of phenothiazine were fed daily beginning four days before inoculation and continued until 28 days after inoculation. The first eggs were recovered from the feces 72 days after inoculation (December 7th) or 29 days after the maximum time when eggs are usually first recovered. This animal never showed any symptoms of parasitism at any time during the experiment. The results with this animal thus agree with those obtained previously and merely show the effects of the drug on egg production when given in the later part of the prepatent period.

No. 229 was inoculated with 106,860 nodular worm larvae on October 11, 1951. The feeding of 1.5 grams of phenothiazine per day was started seven days before inoculation, and was continued in the milk after the animal quit eating the grain. On October 15th he refused the grain and developed a very bad diarrhea, the discharge containing a large amount of mucus. On the 16th, 17th and 18th he refused almost all the grain ration, but continued to drink the milk in which the phenothiazine was mixed. On the morning of October 19th he did not get up. The fecal sample was very watery and contained a quantity of blood. About 10:30 a.m. the animal died. At post mortem examination the walls of the anterior one-half, or a little more than one-half of the small intestine appeared normal, except for numerous pinpoint hemorrhagic spots on the last three or four feet. The contents of this anterior portion of the small intestine were normal. The hemorrhagic spots became more numerous and more conspicuous in the posterior half of the small intestine. The position of many of the hemorrhagic spots was evident on the outside of the intestinal wall. The wall of the posterior seven to eight feet had a very large number of these hemorrhagic areas opposite the attachment of the mesentery. This portion of the intestinal wall was very edematous, thickened and very friable and the contents were very bloody. The walls of the caecum and anterior four to five feet of the large intestine just

beyond the iliocaecal value had very numerous hemorrhagic spots similar to those in the small intestine. The wall was thickened and the contents bloody. Fecal examinations on September 24, October 1 and 10th, demonstrated the absence of all parasitic ova.

No. 233 was inoculated on October 20, 1951 with 99,000 nodular worm larvae. The feeding of 1.5 grams of phenothiazine was begun on October 15th and continued in the feed and then in the milk after he refused the grain ration, until he quit eating altogether. He refused his grain on October 22nd and ate very little grain from then until death. On October 24th the first diarrhea was noted. The discharge was very watery and contained much mucus until his death. On October 26, 27 and 28 refused all feed and drink and death occurred some time during October 28th. At the post mortem examination the condition of the small and large intestine was the same in general as that of No. 229.

The results of the experiments on 229 and 233 indicate very forcibly that phenothiazine has no effect on the developing larvae in the early stages or on the course of the infection.

Summary and Conclusions

One and one-half grams of phenothiazine fed daily during the first 28 days or from the 14th to the 28th day after inoculation, delayed the appearance of nodular worm eggs from 10 to 27 days after the maximum time when eggs are usually recovered from the feces of calves. The drug also greatly reduced the number of eggs recovered for a considerable time. While some animals produced small numbers of eggs, others failed to produce any eggs during the period of observation.

One animal that did become positive (No. 221) developed a sufficiently high egg count to permit larvae to be cultured for additional inoculations. Thus, such animals serve as a source of infection unless continuous feeding is practiced.

Feeding the drug at the rate of 1.5 grams per day during the first two weeks of the prepatent period, did not interfere with the normal development of the larvae or with egg production (Nos. 204 and 209).

Symptoms and death were not prevented when the drug was fed at the rate of 1.5 grams per day from the beginning of the prepatent period.

The continuous feeding of phenothiazine in small amounts is a measure to be employed to reduce nodular worm infections on premises known to be heavily contaminated. There is no evidence of any therapeutic value brought forth by the above described experiments. Neither is there any prophylactic value indicated under conditions of heavily contaminated premises, but some protection is promised for the next generation of animals.

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Enterobius vermicularis Infection in Patients with Poliomyelitis

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The epidemiology of poliomyelitis has been considered to resemble in some ways that of infection with pinworm, *Enterobius vermicularis*. The possibility that nematode eggs or larvae might serve as one means of transmission of the virus was suggested by Thompson (1948), and since that time has been discussed in some detail by Gonzáles Castro (1950) and Gonzáles Castro and Manãs Montalvo (1952, 1953).

Our interest in the problem prompted a survey during the summer of 1950 to obtain data on the incidence of pinworm in patients with poliomyelitis in two hospitals in the District of Columbia. The survey included reported cases of both paralytic and nonparalytic type poliomyelitis, a total of 252 cases; all were newly admitted to the hospital. Although it was recognized that data from such a survey would neither prove or disprove the possibility of virus transmission by the nematode, it was believed that the information, not hitherto available, as to the incidence of pinworm in a representative group of such patients would be important for evaluating the problem and possibly planning other approaches.

Permission to gather the data and to publish the findings was granted by Dr. Montgomery Blair of Children's Hospital and by Dr. L. E. Hoeck of Gallinger Hospital. Their cooperation and that of their respective staffs is greatly appreciated.

It was decided that no "control" group would be examined. The difficulties of obtaining what might be regarded as a matched random sample are obvious. However, for patients hospitalized with other illnesses some comparative data are available for the younger age groups from an earlier survey made at one of the same hospitals by E. C. Jones (1942). Data are available also from examinations made by the writer on a small series of necropsy specimens obtained from both hospitals (1941).

The NIH cellophane swab was used in the present survey. The relative safety in handling the material, from the time the swabs were used at the hospital to their examination at the Laboratory of Tropical Diseases at Bethesda, was one factor in the decision to use this swab rather than any of the adhesive cellophane swabs now in use. The hospital routine of taking rectal temperatures, the frequent presence of oil or other lubricant on the perianal region, and the lack of information as to the effect of acute illness on the migration of the worms complicated the choice of swab and of the best time for its use. The swabs were used by the nurses on duty in the isolation wards, in most cases, on successive nights before midnight temperatures were taken. One to 7 swabs, rarely more, were used on each patient. All swabs were examined by the writer. It was found that a weak solution of the wetting agent "Alconox" facilitated examination of the cellophane.

Results of examinations are presented in Table 1. Of 252 persons examined, 50 or 20 per cent were positive on one or more swabs. If one includes

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as negative only those persons for whom at least 4 negative swabs were obtained, there were 50 of 211 or 24 per cent positive. Seven of 52 or 13 per cent of the Negroes were positive, and 43 of 200 white persons or 22 per cent were positive. Thirty-three of 139 males or 24 per cent were positive and 17 of 113 females or 15 per cent were positive. As indicated in the table, most of the patients were in the 0 to 12 years age group; the greatest prevalence, 24 per cent positive, also occurred in this group as compared to 8 per cent in the older group of which more than half were adults. A lower incidence is noted for Negroes in the younger, but not in the older group.

An estimated corrected prevalence for some of these groups was made by Mr. N. Mantel (Analytical Statistician, National Institutes of Health) on the basis of "swab efficiencies" derived from the complete data for each group. The highest swab efficiency obtained was for the white males of the 0 to 12 years group (53±4 per cent); for other groups it was near 20 per cent. The same white male group provided the highest estimated prevalence, 36 per cent, adjusted from 31 per cent observed positive. The estimated prevalence for white females of the same age groups was 27 per cent, adjusted from 19 per cent observed positive; for Negro males, 23 per cent, adjusted from 16 per cent; and for white females of the older group it was 18 per cent, adjusted from 12 per cent. Data from other groups did not justify such analysis.

In the survey by E. C. Jones mentioned previously, the findings were similar to these for white children 0 to 12 years (15 of 60 were positive), but fewer Negro children were found positive (2 of 60). In the necropsy series, pinworms were recovered from 10 of 24 specimens from white children and from 11 of 48 specimens from Negro children. Insofar as comparisons can be made the findings in the present survey do not appear to be markedly different from those on children hospitalized for illnesses other than poliomyelitis.

Some thought was given to the matter of the time necessary for development of the pinworm to the mature egg-depositing stage as compared to that possibly required for development of the acute stage of poliomyelitis. The problem could not be studied in detail. An original plan to make repeated series of swab examinations of all patients negative on the first series did not prove to be practicable. Only six negative patients were re-examined, the second series of swabs being taken two to five weeks after the first swab examination. None of these patients was positive on later examination.

Another matter of some interest was the pinworm infection in poliomyelitis patients from the same household. Only a few such cases were included in the present survey. Of five pairs, both were positive in two cases, both were negative in two cases and in the fifth one was positive and one negative.

TABLE 1.—Infection with *Enterobius vermicularis* in reported cases of poliomyelitis (number positive over number examined)

Age (years)	Race	Sex		Totals					
		Male	Female	White	Negro	Male	Female	All cases	Per cent positive
0 to 12	White	28/88	11/58						
	Negro	4/25	2/16	39/146	6/41	32/113	13/74	45/187	24
Over 12	White	0/21	4/33						
	Negro	1/5	0/6	4/54	1/11	1/26	4/39	5/65	8
All ages (0 to 45)									
Per cent positive				43/200 22	7/52 (13)	33/139 24	17/113 15	50/252 20	

Considering all the poliomyelitis patients as one group the incidence of pinworm infection is judged to be relatively low since only 20 per cent gave evidence of migrating worms, and moreover, on the basis of the number of positive swabs per person, only a few cases appeared to be heavily parasitized. The data demonstrate that pinworm eggs are disseminated from patients at times when the virus may also be present in the intestinal tract. Possibly more intensive follow-up studies, such as those on the six patients referred to previously, might give more information on early pinworm infections not detectable by swab examination made at the time of poliomyelitis diagnosis. Household and other group studies might also be profitable. It is our opinion, however, that an experimental attack on the problem will be necessary to obtain definite information as to the possibility of a carrier relationship between the nematode and the virus.

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Two New Rhopalocercariae (Gorgoderinae) Parasitic in Lake Erie Mussels

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Two gorgoderid cercariae have been found to develop in sporocysts in the viscera of two species of clams collected from Lake Erie. With bodies characteristic of the Gorgoderinae, these new species possess the specialized tail characteristic of the rhopalocercariae. They are differentiated from known species by the number of penetration glands, number and distribution of sensory papillae, size of body, and the shape of the transformed tail. Pelecypod molluscs had been examined for 18 months before cercariae of this

type were found. This would seem to indicate that these new species are either very localized or exhibit a seasonal shedding of cercariae.

One of the first gorgoderid rhopalocercariae described was *Distomum duplicatum* v. Baer, 1827, which was given names by several of the early investigators. Diesing, 1850, renamed it *Rhopalocerca tardigrada* and Pagenstecher, 1857, thought it was the larval stage of *Distomum cygnoides,* thus correctly recognizing its affinities to the gorgoderid tredmatodes.

Concerning von Baer's *Cercaria duplicata,* Nybelin, 1926, thought that three species had been confused: 1) *C. duplicata* Wagener, 1857, was described with a Y-shaped excretory system and a pharynx and, therefore, could not be placed in the Gorgoderidae; 2) he thought that *C. duplicata* Reuss, 1903, is synonymous with *C. duplicata* v. Baer, 1827, and he stated that the drawing by Pagenstecher, 1857, of *C. duplicata* was in error by depicting this form with a pharynx.

Five more rhopalocercariae were described by Fischthal, 1951, from unionids from rivers in Michigan and New York. He gave no consideration to the adults of these distomes, but stated that, on the basis of the excretory system, they belonged to the Gorgodera-Catoptroides group. The cercariae studied by Fischthal, 1951, were *C. micromyae* from *Micromya iris* and *Alasmidonta marginata* in the Huron River, Michigan, *C. catatonki,* parasitic in *Strophitus undulatus quadriplicatus* in Catatonk Creek, New York, *C. honeyi* in the viscera of *Anondontoides ferussocianus* and *Alasmidonta calceolus* from Honey Creek, Michigan, *C. pyriformis* in *Micromya iris* from the Huron River in Michigan, and *C. filicauda* from *Elliptio dilatatus* in the Huron River.

J. Leidy, 1858, reported from North America *Rhopalocerca tardigrada* Diesing from the mantle of *Anodonta fluviatilis* and specimens also from *A. lacustris.* As reported by Fischthal, 1951, in all probability Leidy was concerned with a new North American species. Fischthal, 1951, gives an excellent consideration of the morphology of the gorgoderid cercariae and postulates phylogeny of the group on the basis of morphology superimposed on the evolution of the pelecypod hosts.

Methods

Cercariae were studied alive, in stained whole mounts, and in sections. Cercariae to be studied alive were removed from sporocysts and from transformed tails both before and after encystment. In some cases, the tail separated from the body before encystment, leaving the cercarial body free for study. Vital stains used were: nile blue sulphate, neutral red, neutral violet, methylene blue, and toluidene blue. Whole mounts were stained with Harris' haemotoxylin and Semicohn's carmine, while sectioned material was stained with Harris' haemotoxylin, Heidenhain's iron haemotoxylin, and Romeis' Kresazan. The fixatives used were: A.F.A., Sanfalice's, Carnoy's, and Schaudinn's. This study was financed by the Ohio Division of Wildlife. The author is indebted to Dr. E. W. Price for checking the availability of the specific names.

Mussels to be examined for emerging cercariae were collected from Lake Erie off a mud bottom at depths ranging from 20 to 35 feet. One species of rhopalocercaria was found to develop in *Lampsilis siliquoidea,* and upon examination it was found to be a new species closely related to *Cercaria pyriformis* Fischthal, 1951, but differing in several respects.

Cercaria pyriformoides sp. nov.
(Figs. 1-6, 12, 18, 19, 21, 22, 24, and 25)

SPECIFIC DIAGNOSIS: apharyngeate, rhopalocercariae lacking a stylet and possessing the characters of the subfamily Gorgoderinae Looss, 1899. The average body measurements in millimeters of ten specimens fixed in warm A.F.A. and mounted in glycerine gel are: length of body 0.732 (0.546-1.28), width of oral sucker 0.0730 (0.571-0.871), width of anterior end at level of oral sucker 0.0909 (0.0689-0.171), distance between oral sucker and acetabulum 0.237 (0.138-0.408), length of esophagus 0.0703 (0.0319-0.144), width of acetabulum 0.0886 (0.0760-0.0989), width of body at level of acetabulum 0.137 (0.0961-0.150), distance between acetabulum and end of body 0.321 (0.231-0.483), distance between acetabulum and excretory bladder 0.0454 (0.0180-0.108), length of excretory bladder 0.274 (0.213-0.390), width of excretory bladder 0.0375 (0.0240-0.0450), width of posterior end of body 0.0729 (0.0571-0.0820), length of tail 0.385 (0.286-0.442), width of tail 0.109 (0.0808-0.175), distance between end of gut and end of worm 0.0423 (0.0320-0.0509). Body shape roughly spatulate with widest portion just posterior to acetabulum. Tail deeply plaited longitudinally distending to a robust pyriform shape after emergence and transformation (Figs. 24 and 25). Numerous, unicellular cystogenous glands, opening to the exterior on the ventral side, found throughout much of the body (Fig. 19). The slender, pyriform gland extends less than halfway toward dorsal surface and the large nucleus, possessing a large nucleolus and small chromatin particles, is located eccentrically in the middle third of the gland. These glands when living appear hyaline, staining only slightly with neutral red, methylene blue, and neutral violet. Fixed glands very slightly granular, staining readily with eosin. Other ellipsoildal, unicellular cystogenous glands crowded around bladder opening into it by a short, slender duct. Fixed glands irregularly granular, rarely staining with eosin. Large nucleus located distally. Bifurcate intestine extends to posterior part of body. Wall of esophagus slightly muscular. Intestinal lumen large and irregular. Tubular excretory bladder opening to exterior through a subterminal pore which lies in the bottom of a small crater surrounded by sensory papillae (Fig. 2). Sphincter present. Bladder extends anteriorly to region of genital glands. Common collecting ducts arise from bladder subterminally, reflexing in the region anterior to the penetration glands. Common collecting ducts bifurcate in region of acetabulum (Fig. 18). Flame cell formula determined to be: $2[(5+7+6)+(7+7+7)]$. Suckers well developed. Bilobed "brain" dorsad to esophagus. Eleven pairs of penetration glands in region of intestinal bifurcation. Ducts with large lumens extend to posterior of oral sucker where a progressive reduction in diameter occurs until the pores opening to the exterior are minute. Glands and ducts filled with coarsely granular material staining readily with nile blue sulphate, eosin, neutral red, azocarmine, and aniline blue. Large nuclei present which are similar to other nuclei in glands of this worm. Reproductive system well-developed at this stage with most of the component parts present. Asymmetrically placed testes on opposite sides of bladder. Ovary and oviduct on right side. Vitellaria posterior to acetabulum, anterior to ovary, and lateral to median. Fundaments of genital ducts present as a chain of cells leading from region of oötype dorsal to the acetabulum to the genital pore. Prostate gland, seminal vesicle and receptaculum uterinum not differentiated. Sensory papillae found on all body surfaces (Fig. 5). Some

papillae have a single seta. Thirty-four papillae on oral sucker with eight of these inside the mouth and the remainder mostly on the anterior half of the sucker. (Fig. 6). Acetabulum with six "double" papillae arranged in a hexagonal pattern (Fig. 3) Seven papillae on posterior half of acetabulum; three large ones on the posterior margin and four others which are smaller are lateral to the marginal papillae. Ten pairs along the postacetabular margin, the last three pairs of which possess setae. Cercariae develop in ellipsoidal sporocysts found in the viscera of mussels (Figs. 1 and 13). One to three cercariae at different stages of development in each sporocyst.

First Intermediate Host: *Lampsilis siliquoidea*

Site: Viscera in the region of the gonads.

Type Locality: Lake Erie, Ottawa County, Ohio; U.S.A.

Type Specimens: Paratypes in Helminthological Collection, U. S. National Museum. No. 48707

Cercaria pyriformoides possesses the general characters of all the rhopalocercariae and is especially closely related to *C. pyriformis* Fischthal, 1951. The species at hand differs from this one of Fischthal's, however, by the presence of either two or three papillae on the posterior margin of the acetabulum, by possessing either six or seven rather than five papillae on the postacetabular dorsum, and by the body size.

Another rhopalocercaria was found to develop in a single specimen of *Anodonta grandis* from Lake Erie. This mussel was collected at a depth of about 30 feet from a mud bottom. It was one of 59 of this species examined for emerging cercaria. The host species constitutes about 1 percent of that part of the unionid population whose smallest dimension is slightly more than an inch (the size of the mesh on the dredge used for collecting).

Cercaria anodontae sp. nov.
(Figs. 7-11, 14, 20, and 23)

Specific Diagnosis: apharyngeate, rhopalocercaria lacking a stylet and with the characters of the subfamily Gorgoderinae Looss, 1899. Body measurements of ten specimens killed in warm A.F.A. and mounted in glycerine gel are: width of oral sucker 0.0573 (0.0510-0.0630), width of anterior end at level of oral sucker 0.0711 (0.0660-0.0750), length of esophagus 0.0574 (0.0329-0.0841), distance between oral sucker and acetabulum 0.152 (0.129-0.162), width of acetabulum 0.0602 (0.0570-0.0629), width of body at level of acetabulum 0.0964 (0.0930-0.0989), width of posterior end 0.0561 (0.0419-0.0780), length of body 0.549 (0.481-0.586), distance between acetabulum and end of body 0.244 (0.204-0.279), end of gut from end of body 0.0474 (0.0420-0.0629), length of bladder 0.215 (0.183-0.234). Spatulate-shaped body with widest portion just posterior to the acetabulum. Tail roughly boat-shaped, characteristically plaited, becoming, upon distension, ellipsoidal.

Explanation of Plates

A—Acetabulum
AG—Anlagen of genital ducts
AP—Anlage of genital pore
DP—Ducts of penetration glands
E—Esophagus
EB—Excretory bladder
EP—Excretory pore
I—Gut
O—Ovary
OD—Oviduct
OS—Oral sucker
P—Penetration glands
T—Tail
TE—Testes
V—Vitellaria

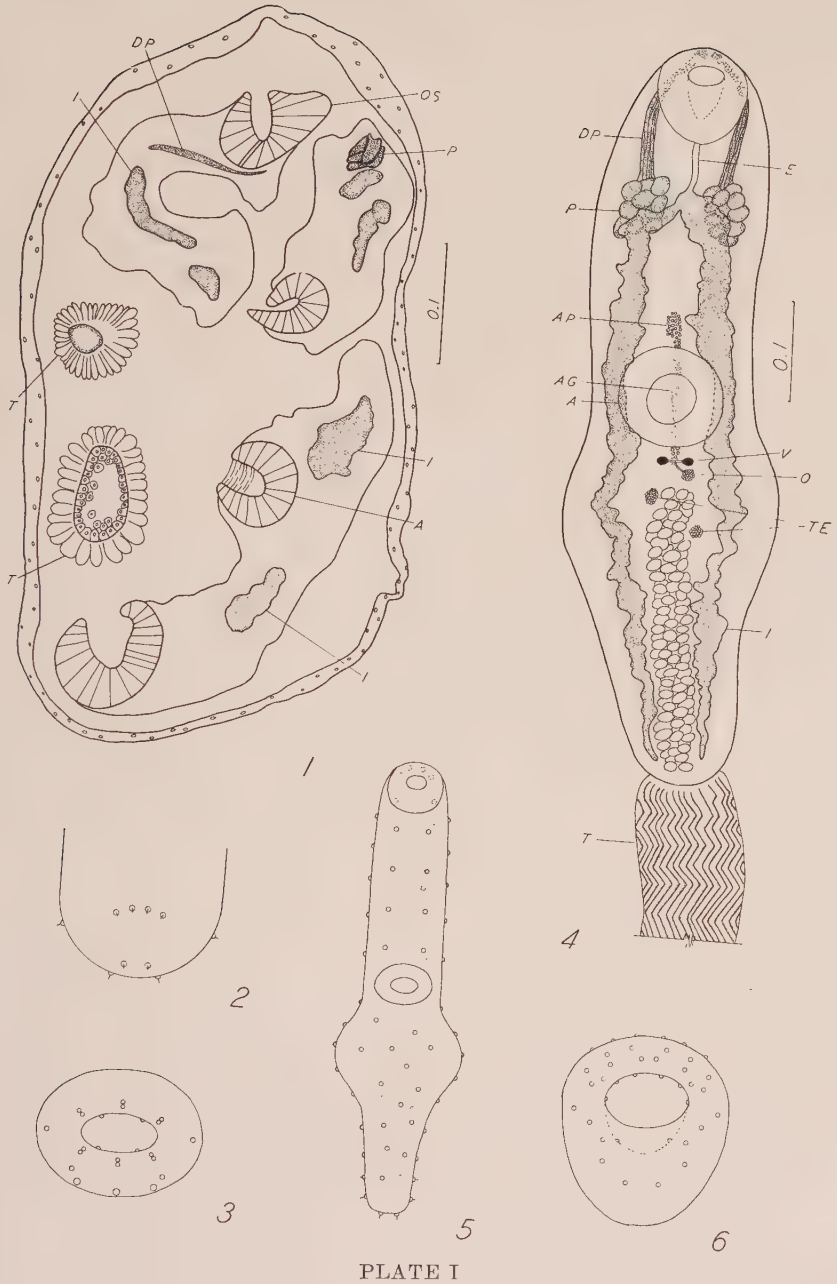

PLATE I

1. Section of daughter sporocyst of *C. pyriformoides* sp. nov. drawn with the aid of a microprojector.
2. Sketch of arrangement of sensory papillae on the posterior ventrum of *C. pyriformoides.*
3. Sketch of arrangement of sensory papillae on acetabulum of *C. pyriformoides.*
4. Whole mount of *C. pyriformoides* drawn with the aid of a microprojector. Fine details were obtained from living specimens and sections.
5. Sketch of arrangement of sensory papillae on body surfaces of *C. pyriformoides.*
6. Sketch of arrangement of sensory papillae on oral sucker of *C. pyriformoides.*

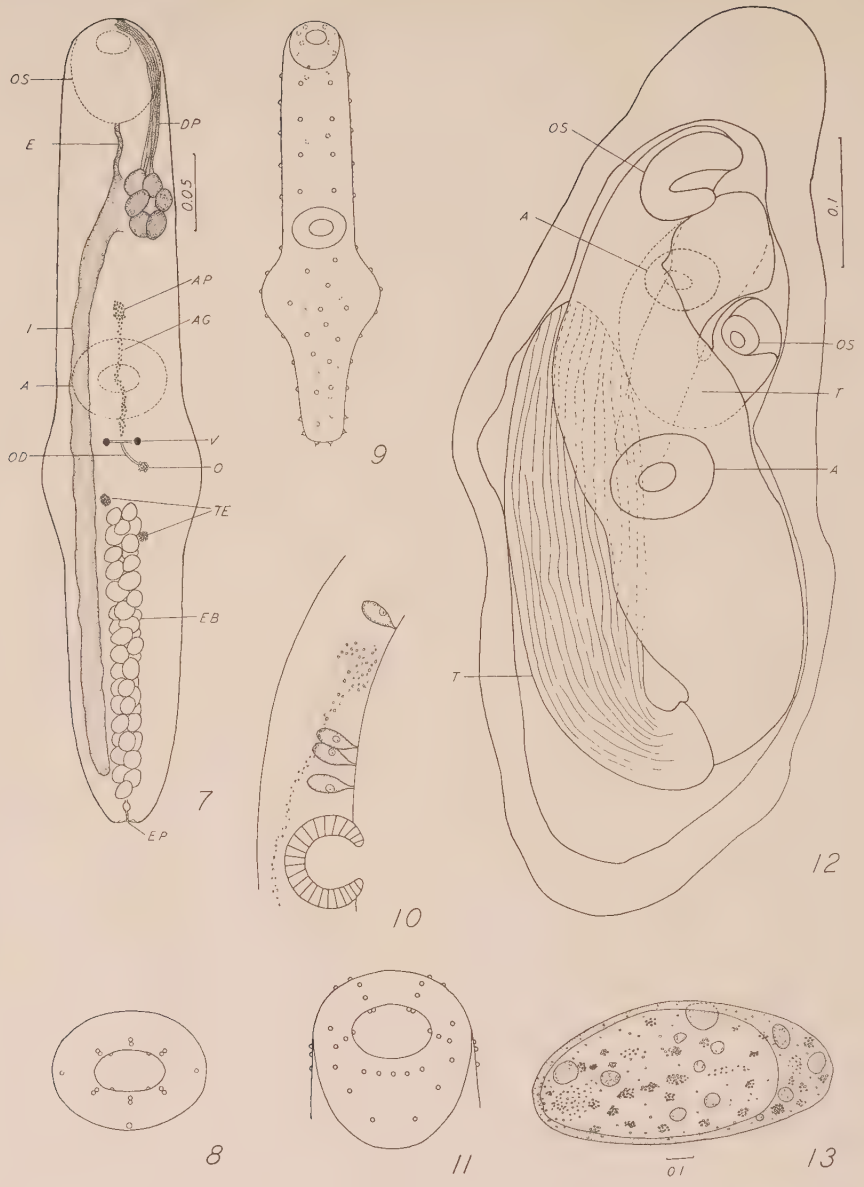

PLATE II

7. Composite drawing of whole mount of *Cercaria anodontae* sp. nov. drawn from information gained from sections, living specimens, and stained whole mounts.
8. Sketch of the arrangement of sensory papillae on acetabulum of *C. anodontae.*
9. Sketch showing the arrangement of sensory papillae on the body surfaces of *C. anodontae.*
10. Sketch of saggital section of *C. anodontae* showing cystogenous glands and fundamentals of the terminal genitalia.
11. Sketch showing arrangement of sensory papillae on the oral sucker of *C. anodontae.*
12. Drawing of whole mount of a sporocyst with two cercariae of *Cercaria pyriformoides;* one quite immature and the other almost mature. Drawn with the aid of a microprojector.
13. Drawing of a very young sporocyst (living) of *C. pyriformoides.* Drawn with the aid of a camera lucida.

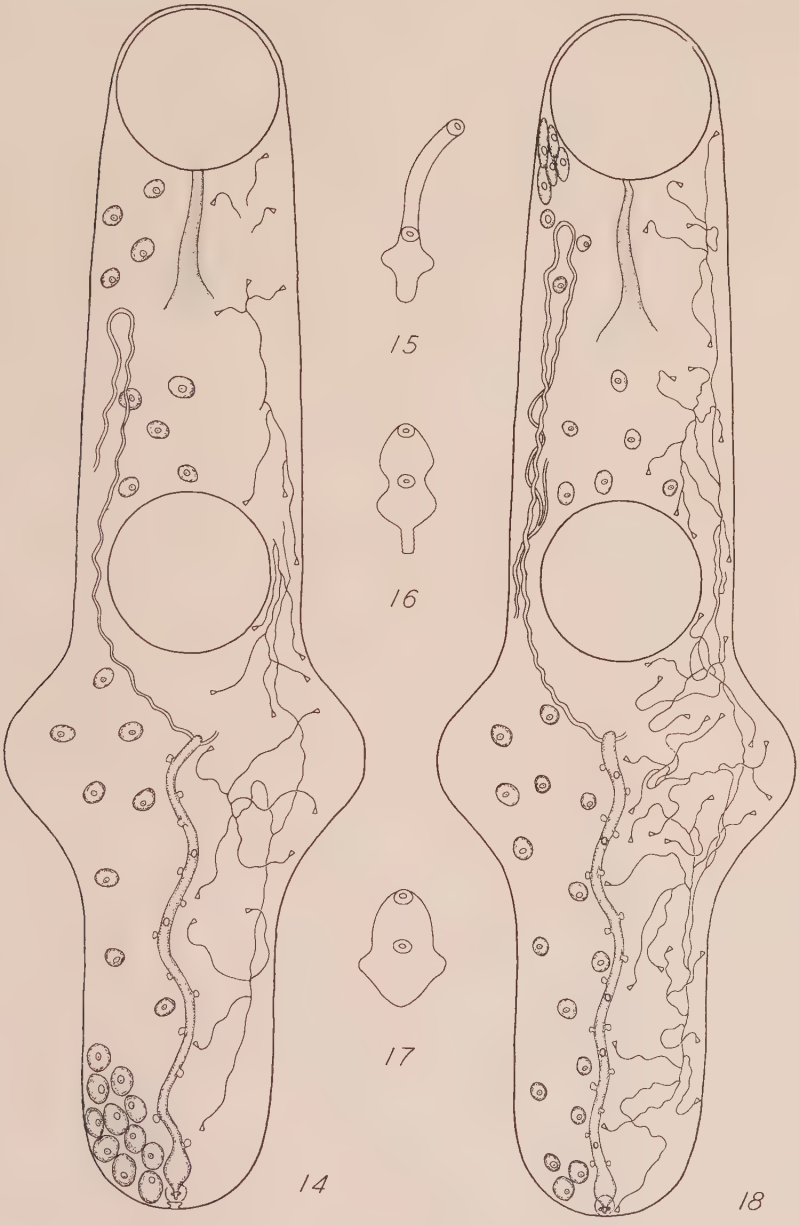

PLATE III

14. Schematic drawing of *C. anodontae* showing part of the excretory system and the distribution of some unicellular glands.
15-17. Sketches showing body shapes of living cercariae.
18. Schematic drawing of *C. pyriformoides* showing the excretory system and the distribution of some unicellular glands.

PLATE IV

All figures are photomicrographs.

19. A saggital section of the anterior half of *C. pyriformoides* showing cystogenous gland opening to ventral surface.
20. Vaseline mount of a moribund specimen of *C. anodontae* stained with neutral red.
21. Whole mount (unstained) of *C. pyriformoides* mounted in glycerine jelly.
22. Metacercaria of *C. pyriformoides* taken of a living, encysted worm lying in lake water.
23. Metacercariae of *C. anodontae* taken of living, encysted worms lying in lake water.
24. Transverse section of the tail of *C. pyriformoides* showing the large cells located inside.
25. Tail of *C. pyriformoides* mounted in glycerine jelly.
26. Whole mount of living *C. pyriformoides* flattened in a Vaseline mount and stained with neutral red.

Tail connected to body by a broad base. Bladder tubular, terminating in the region of the genital glands and surrounded by large, ellipsoidal, unicellular cystogenous glands. Fixed glands contain more or less granular cytoplasm and open into the bladder by a short, slender duct. Other glands, interpreted as cystogenous, present throughout the body and opening to the exterior ventrally with hyaline cytoplasm when living, staining slightly with neutral red. Fixed glands stain readily with eosin. Intestine bifurcate extending to posterior region of body. Esophageal wall slightly muscular. Excretory bladder tubular, opening to the exterior through a small subterminal pore. Anterior end of bladder in region of genital fundaments. Sphincter present. Excretory system not completely determined. Common collecting ducts reflex in region of intestinal bifurcation. Extent of excretory system observed shown in Fig. 14. Reproductive glands differentiated excepting Mehlis' and prostate. Testes on opposite side of bladder lacking symmetry. Oviduct and oötype present. Vitellaria posterior to acetabulum, anterior to ovary, and lateral to median. Fundaments of genital ducts present as line of cells running from oötype, dorsal to acetabulum, to anlage of genital pore. Terminal genitalia not entirely differentiated. Thirty-three papillae on oral sucker with three pairs inside mouth (Fig. 11.) Three pairs sensory papillae along lateral margin of oral sucker, four pairs on preacetabular ventrum, six on preacetabular dorsum. Postacetabular ventrum with 14-16 papillae and postacetabular dorsum with five papillae (Fig. 9). Acetabulum with six double papillae arranged in a hexagonal pattern. Posterior margin with a single, large papilla. Seven pairs of penetration glands in region of intestinal bifurcation. Gland contents coarsely granular, staining with nile blue sulphate, eosin, and neutral red. Ducts with large lumens extend to posterior of oral sucker where they start to become reduced in size. Opening to exterior by minute pores lateral to median line. Large nuclei present which are similar to the nuclei of other glands in this distome. Bilobed "brain" dorsal to esophagus.

FIRST INTERMEDIATE HOST: *Anodonta grandis*

SITE: Viscera in region of the gonads.

TYPE LOCALITY: Lake Erie, Ottawa County, Ohio, U.S.A.

TYPE SPECIMENS: Paratypes in Helminthological Collection, U. S. National Museum. No. 48709

Cercaria anodontae possesses the characters common to the rhopalocercariae, but it does not appear to be closely related to any of the species which have been described. It has the least number of penetration glands described for this group and parasitizes a host which is new for the recent descriptions of species.

Most of the cercariae in this subfamily appear to contain unicellular cystogenous glands surrounding the bladder. As pointed out by Fischthal, 1951, Goodchild, 1943, was incorrect in stating that non-stylet cercariae "lack definitely organized cystogenous gland cells around the excretory bladder." Miller, 1935, Sinitsin, 1905, Krull, 1935, Goodchild, 1939, and Coil, 1953, have noted such glands in gorgoderid cercariae. The formation of the cyst and the disappearance of the glands in the rhopalocercariae and the macrocercariae differs greatly. In the rhopalocercariae studied here, the cyst is formed within 24 hours (depending on temperature) after emergence from the host. In an examination of the metacercaria of this age, the cystogenous glands surrounding the bladder could not be demonstrated, while in the case of *C. eriensis* Coil, 1953, and the forms described by Sinitsin, 1905, the cyst

TABLE I

In the species described above there several unicellular glands which give various reactions to stains. In no instance was there a metachromatic reaction with toluidene blue.

Location of glands	Reaction of cytoplasm to stain
Surrounding bladder	Very irregular; stain sometimes with eosin and haemotoxylin, but usually do not stain.
Lateral to oral sucker	Eosin, neutral red (sl.), neutral violet, and methylene blue (sl.).
Distributed throughout the body; opening to the exterior on ventrum.	Eosin, aniline blue, orange G, neutral red (sl.), neutral violet, methylene blue (sl.), toluidene blue and the Hotchkiss reaction.
Posterior end lateral to the bladder.	Eosin, aniline blue, orange G, neutral red, (sl.), neutral violet, methylene blue (sl.) and toluidene blue.
Region of Intestinal bifurcation.	Eosin, azocarmine, neutral red, aniline blue, nile blue sulphate.

is formed more slowly and the cystogenous glands are slow to deteriorate. Indeed, the disintegration is never completed in the body, but after some reduction, the cells are sloughed off into the lumen of the bladder, from which they pass to the exterior.

The glands found throughout the bodies of some gorgoderid cercariae (*C. eriensis, C. anodontae,* and *C. pyriformoides*) appear to be a little known characteristic of cercariae of this family. Goodchild, 1943, noted the formation of two cyst walls in *Phyllodistomum solidum,* but did not mention the source of the respective layers. Vickers, 1940, described large cells in the anterior region of *C. macrocerca,* and Goodchild, 1943, suggested that these cells may be the source of the secondary cystogenous material. However, Fischthal, 1951, commenting on Goodchild's interpretation of the glands described by Vickers stated: " . . . staining does not preclude the possibility that the cells described by Vickers as cystogenous glands may not be additional penetration glands, for it has been noted in *C. Spirorchis parvus* (Spirorchiidae) by Wall (1941b), and in *C. Leucochloridiomorpha constantiae* (Brachylaemidae) by Allison (1943), that some of the penetration glands were eosinophilic, others basophilic." The rapid disappearance of these glands in *C. eriensis* in contrast to the slow disintegration of the glands around the bladder indicates that these glands probably form the first cyst wall.

Lateral to the oral sucker are cells which may be either cystogenous or cephalic glands. They are very similar to the glands described above, but differ slightly in structure and reaction to stains. In the posterior region, lateral to the bladder, are unicellular glands which are interpreted as cystogenous in nature. In the living *C. anodontae,* they are numerous and appear very similar to the glands surrounding the bladder, but in the case of *C. pyriformoides,* they are fewer and resemble more the cystogenous glands found throughout the body.

It is the opinion of the author that the cystogenous glands scattered through the body have been overlooked by other investigators in their studies of gorgoderid cercariae. The probability of such glands arising independently in closely related species appears to be slight. Furthermore, they have been observed in every gorgoderid cercaria studied by the author.

The penetration glands, usually lying in the region of the intestinal bifurcation, are filled with a coarsely granular material. In the rhopalocercariae, these glands are well developed, although they are not utilized, evidently, to gain entrance into a second intermediate host. Fischthal, 1951, noted that the cercariae discharged one pair of penetration glands while leaving the first intermediate host. It was not determined whether the species at hand utilize a single pair of penetration glands. The number of glands could be determined with certainty only by counting the ducts where they open to the exterior.

The constancy of the occurence of sensory papillae is open to question. In *C. pyriformoides*, the papillae on the posterior margin of the acetabulum varied as follows: five specimens had two and seven specimens had three. This was also found to be true of the papillae on the postacetabular dorsum, (three specimens had six papillae and three specimens had seven).

The excretory system of *C. anodontae* was not completed in spite of assiduous efforts. It was noted, however, that it is of the stenostoma type, and that it is typical of the other types which have been described for the rhopalocercariae. (The excretory system was probably completely determined, however, for *C. pyriformoides*). The reflexion of the primary collecting ducts in *C. pyriformoides* occurs anterior to the penetration glands in the region of the esophagus. This condition is very similar to that found in *C. eriensis*. In all other known instances, the reflexion occurs more posteriorly It is also of interest to note that *C. pyriformoides* possesses the same number of accessory collecting ducts as *C. eriensis*.

As a character, the importance of the loop of the primary collecting ducts is largely unknown. Dubois, 1929, and Sewell, 1922, have discussed groups delimited by this character; however, these categories are very heterogeneous when other important characters are considered. The size of the category to be delimited by this character can be determined only after morphological studies of all stages of the life cycle and after comparison with other relatively stable structures. In the Gorgoderinae (Byrd et al, 1940) and the Allocreadiidae (Seitner, 1951, and Hopkins, 1934, 1937) both the mesostoma and stenostoma types of excretory system have been reported. The size of the category, therefore, probably is suprageneric and smaller than a superfamily in view of the fact that several investigators have suggested that these two groups are much too diverse to remain as they are now classified. Furthermore, Fischthal, 1951, depicted the loop as arising quite early in the ontogeny of the rhopalocercariae.

We are, therefore, concerned with the significance of the level of reflexion in *C. pyriformoides*. Is this species more primitive than most of the rhopalocercariae by virtue of the possession of primary collecting ducts which exhibit a reflexion intermediate between the rhopalocercariae and the primitive *C. eriensis?* A consideration of the evidence presented by other structures clarifies this question somewhat. Possibly, in the rhopalocercariae, there is a concomitant reduction in body size, in the number of penetration glands, and in the length of tail. *C. pyriformis, C. filicauda,* and *C. pyriformoides* all have 11 pairs of penetration glands; the first species is the smallest of these

three, and the third is the largest. *C. anodonta,* which possesses only 7 pairs of penetration glands, is the smallest rhopalocercaria described for North America. It would appear, however, that *C. filicauda* is the most primitive of the gorgoderid cercariae on the basis of the tail structure. Its excretory system is not completely known.

On the basis of the evidence available (excretory system, penetration glands, and body size), *C. pyriformoides* is clearly one of the least specialized of the rhopalocercariae, being closely related to *C. pyriformis* and more distantly related to *C. filicauda.*

The life cycle of a rhopalocercaria has never been completed, although it appears obvious. The complete utilization of the cystogenous glands and the absence of a stylet seem to preclude the possibility of a second intermediate host. *C. mitocerca* Miller, 1935, is the only known astyleted, gorgoderid cercaria which utilizes an intermediate host (presumably). On the other hand, cysts are not formed by some brachylaemids, the metacercaria of which have been reported lying free in the host's body.

KEY TO THE RHOPALOCERCARIAE OF AMERICA

A. Cercariae with 11 pairs of penetration glands ..... B

AA. Cercariae with less than 11 pairs of penetration glands ..... D

B. Transformed tail with long posterior filament
Cercaria filicauda Fischthal, 1951

BB. Transformed tail lacking long posterior filament C

C. Five papillae on postacetabular dorsum
Cercaria pyriformis Fischthal, 1951

CC. Six or seven papillae on postacetabular dorsum
Cercaria pyriformoides sp. nov.

D. Nine pairs of penetration glands ..... E

DD. Seven pairs of penetration glands ..... *Cercaria anodontae* sp. nov.

E. Two sensory papillae on posterior margin of acetabulum
Cercaria micromyae Fischthal, 1951

EE. One papilla on the posterior margin of the acetabulum ..... F

F. Five papillae on dorsum posterior to acetabulum
Cercaria catatonki Fischthal, 1951

FF. Seven to eight papillae on dorsum posterior to acetabulum
Cercaria honeyi Fischthal, 1951

The only other species described, *Cercaria duplicata* von Baer, 1827, is readily differentiated from the North American species by the cellular contents of the cercarial tail which is displaced into an eccentric position by the cercarial body, while in the species listed above, the cells are in a symmetrical position at the posterior end of the transformed tail.

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Parasites of Skunks in the Beltsville, Maryland, Area*

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During the period from November 1949 to August 1951, 14 mature skunks, *Mephitis mephitis nigra,* were killed on the premises of the Zoological Division station at Beltsville, Maryland, and examined for parasites. Four were autopsied in June, one each in August, November, December and January, and six in February. The parasites of each animal were classified as to kind, number, degree of development, and location.

Inasmuch as a review of recent literature on helminth parasites of the Mustelidae and a summary of the helminths found in these hosts were published by Erickson (1946), the records mentioned therein will not be repeated. Later, however, Spindler and Permenter (1951) recovered considerable numbers of *Trichinella spiralis* larvae from three of four skunks obtained from the same area in which the present study was made.

Examination of the contents of the digestive tracts indicated the source of

*Thanks are due to the following members of the Zoological Division, Beltsville, Maryland: Dr. Gerard Dikmans, under whose auspices this study was made, and Mr. Allen McIntosh for identification of a specimen of *Ixodes cookei*.

the majority of the parasites. The skunks examined were omnivorous, but insects, particularly adult, and sometimes larval, Coleoptera, and Orthoptera, appeared to be the major items in their diet. Other ingesta were rodents, mollusks and plant material.

Results and Discussion

The principal data are given in table 1. Of the external parasites, engorged specimens of ticks were present in midwinter as well as in warm weather. All ticks except two, recovered in November, were mature. Biting lice, *Neotrichodectes mephitidis,* were sometimes very abundant, more than a thousand in one case. They appeared to be more abundant in winter than at other seasons. Egg cases were noted on the hairs of some hosts in February. One flea, *Ceratophyllus* sp., was caught as it jumped away from its dead host.

Internal parasites occurred in almost every part of the body. *Skrjabingylus chitwoodorum,* a metastrongylid, has a blood-red pigment, primarily present in the body wall, and occurs in the frontal sinuses. Regardless of the time of year, all the worms collected were mature. Perhaps mollusks are the intermediate hosts.

Gongylonema pulchrum occurred partly imbedded in the buccal, pharyngeal and oesophageal mucosae, but primarily partly under the rasping surface of the tongue. One to 40 worms were recovered from each of 13 of the 14 hosts. Mature and immature forms were present at all times of the year. Insects, especially dung beetles, are the intermediate hosts of this parasite.

To the author's knowledge, the skunk has not previously been reported as a host of *Gongylonema pulchrum,* which is also a parasite of ruminants, swine, and other mammals. The dimensions of this parasite vary greatly with the host species. Measurements of four gravid females and six associated mature males recovered from a skunk are given in Table 2. Although the adults were smaller than those from sheep in the same area, the eggs were the same size. Interspecific host transmission experiments, such as those performed by Lucker (1932), showed that the parasite can readily be transmitted from one host species to another. The skunk is, therefore, a possible source of infection for livestock. Since experimental infections with this parasite have not been made at the station for approximately 20 years, the skunk is considered to be a normal host of *Gongylonema pulchrum.*

The species varies in several other respects besides total length. The mature females recovered from the skunk showed a considerable range in the distance of the vulva from the posterior end, and the males had four to six, usually five, pairs of preanal papillae and three to six, usually five, pairs of postanal papillae. The left spicule was pointed at the distal end and varied considerably in length.

Physalopter maxillaris, a spirurid nematode, occurred in the stomach and occasionally extended into the oesophagus. These worms were firmly attached to the stomach wall, apparently feeding on the tissues or their fluids. Infestation was so extensive in one skunk that half the stomach surface was occupied by worms and most of the lumen was filled by them. There was marked hypertrophy of the stomach wall in severe infestations. Skunks autopsied in winter harbored very few mature worms, one to ten, and usually several hundred very small immature worms, whereas those autopsied in June were parasitized by as many as 200 mature worms and very few immature ones. Insects probably are the intermediate hosts of this parasite. All 14 hosts were infested with this species.

TABLE 1. Numbers of Parasites Recovered from 14 Skunks, *Mephitis mephitis nigra*, from the vicinity of Beltsville, Maryland

Parasite		Date of Autopsy and Numbers of Parasites										
		6/12/51	6/14/51[1]	6/22/51[2]	8/9/51[1]	11/14/49	12/13/49	1/15/51	2/19/51	2/20/51[2]	2/21/50	2/21 and 23/51[2]
Skrjabinglyus chitwoodorum	mature	8	10	6 8	0	0	18	6	0	3 0	8	4 17
Gongylonema pulchrum	mature	2	3	3 1	0	0	1	10	5	2	0	20
	immature	8	2	0 0	10	3	0	30	4	5	0	22
Physaloptera maxillaris	mature	200	108	125	1	5	15	1	0	10	5	0
	immature	Few	12	14	39	12	5	Many	10	Many	Many	Many[3]
Oochoristica sp.	mature	30	20	22	0	20	0	0	0	0	0	0
	immature	5	Very Few	0	0	0	3	Many	Many	4	8	50
Ascaris columnaris	mature	0	0	5	16	7	11	2	5	10	1	0
	immature	4	1	14	7	0	10	5	0	20	1	0
Arthrocephalus lotoris		2	3	7	0	0	1	5	3	12	2	5
Molineus patens		5	29	22	0	0	10	3	0	0	0	12
Capillaria sp.		0	0	2	0	0	23	[4]	0	[4] 0	[4]	0
Strongyloides sp.		1	0	0	0	0	0	1	0	0	5	12
Dipetalonema spp.		3	....	0	....	0	4	0	1	0	1	0
Alaria taxideae		2	0	1	0	0	0	12	0	0	0	0
Macracanthorhynchus ingens		0	0	5 0	0	0	0	0	0	0	0	0
Issospora sp.[5]		+	++	++ ++	+	[6]	[6]	+	+	+ +	+	+ 0
Dermacentor variabilis and *Ixodes cookei*		0	3	1	....	3	0	1	1	2	10	0
Neotrichodectes mephitidis[7]		0	....	0	....	+	+	0	+	++ ++	++	0
Ceratophyllus sp.		0	....	0	....	0	0	0	0	1 0	0	0

[1]Only head and digestive tract of animal available.
[2]Totals for two animals, where only one figure is given.
[3]Several hundred in each animal.
[4]Eggs in feces.
[5]+ = few to moderate number of oocysts, ++ = many oocytes.
[6]Not examined for coccidia.
[7]+ = few to moderate number, ++ = large number.

TABLE 2.—Measurements in millimeters of *Gongylonema pulchrum* from the skunk

Item Measured	Four gravid females		Six mature males associated with gravid females	
	Range	Average	Range	Average
Total length	22.3 -27.8	25.3	11.7 -18.7	16.0
Pharynx length	0.044- 0.050	0.046	0.037- 0.051	0.044
Pharynx width	0.007- 0.010	0.009	0.007- 0.009	0.008
Anterior end to cervical papillae	0.13 - 0.16	0.15	0.13 - 0.15	0.14
Anterior end to last cuticular plaque	0.87 - 1.05	0.96	0.58 - 0.89	0.77
Anterior end to end of oesophagus	4.64 - 5.16	4.96	3.54 - 4.33	4.02
Body width at base of oesophagus	0.17 - 0.18	0.18	0.13 - 0.18	0.15
Posterior end to vulva	1.38 - 2.29	1.70		
Dimensions of eggs	0.053- 0.065 × 0.032- 0.035	0.059 × 0.034		
Anus to tip of tail	0.17 - 0.21	0.19		
Body width at anus	0.079- 0.093	0.082		
Length of left spicule			4.60 - 9.10	7.47
Length of right spicule			0.096- 0.118	0.106
Length of gubernaculum			0.069- 0.100	0.087

The most conspicuous intestinal nematode parasite was *Ascaris columnaris*. One to 30 specimens were present in 12 of the 14 animals. No marked seasonal trend in infestation was noted. However, two of the four animals autopsied in June contained only immature worms, and one each in November and February contained only adults, whereas the eight other infested skunks, autopsied from June to February, harbored mature adults, which averaged about 7.5 cm. in length, as well as immature worms.

The other nematodes in the intestine were small and usually few in number. Twelve of the 14 skunks were infested with the hookworm, *Arthrocephalus lotoris,* one to 12 adult worms being present in each animal. Of the 15 females recovered in winter none were passing eggs, whereas three of the five recovered in June were gravid. Eight skunks harbored the trichostrongylid, *Molineus patens*. Three to 29 adult worms were recovered from each host. In general, greater numbers were recovered in June than in winter. Two to 23 adult *Capillaria* sp. were recovered from the stomach and intestine of two of the skunks, and eggs were found in the feces of three other skunks. This genus has not previously been reported from skunks. There were one to 12 mature *Strongyloides* sp. in each of five of the skunks.

Of the parenteral parasites, two complete filarids, one female and one male, and two incomplete specimens were recovered from one skunk and one to three fragments, of different individuals, were recovered from three other skunks. Because of the time required to examine each organ separately, other than the digestive tract and the sinuses, the larger organs were cut up and the combined washings from them and the body cavities examined together. This procedure accounts for the fact that most of the filarids were incomplete. The entire female was found free in a dish of water containing the uncut thoracic organs. Apparently two species were represented. One has a bulbous head with conspicuous cephalic papillae, no buccal vestibule, and a slight constriction in the neck region. The other species also has a bulbous head but no constriction in the neck area, the cephalic papillae are not as conspicuous, and a small buccal vestibule is present. The former species is more slender than the latter. The complete female belonged to the latter species, and had the following measurements: total length 95 mm.; body width 0.25 mm.;

length of oesophagus 3.5 mm.; anterior end to vulva 1.65 mm.; anus to tip of tail 0.41 mm. There are a pair of small lateral appendages and a slightly larger medial one at the end of the tail, which curves dorsad. Microfilariae were present in the uterus. Those from the uterus of a fragment of the same species measured 220 x 3 micra. Two anterior fragments, which appear to be parts of males of this species, measured 0.17 mm. in width and the distance from the anterior end to the end of the oesophagus was 2.6 mm. The complete male belonged to the former species. It had the following measurements: total length 27 mm.; body width 0.07 mm.; anus to tip of tail 0.10 mm.; length of long spicule, which was of the whip type, 0.42 mm.; length of short spicule 0.085 mm. The tail was coiled and had four pairs of preanal papillae and five pairs of postanal papillae. Both species of filarids appear to belong to the genus *Dipetalonema,* which has not been reported from skunks.

The blood of two of the skunks harboring these filarids was examined for microfilariae. In both cases they were present in considerable numbers. Sections of mircofilariae observed in the blood vessels, in stained sections of lung tissue, showed no sheath. Nuclei were abundant, there being two nuclei across the width of the body for most of the length, and the last nucleus was compressed in the attenuated end of the body. These parasites are transmitted by blood-sucking insects.

Oocysts, assigned to the genus *Isospora,* were found in the feces of 12 animals examined for Coccidia. Their dimensions were 17.6-22.1 x 16.2-19.1 micra.

A tapeworm, *Oochoristica* sp., occurred in the intestine of 13 of the 14 hosts. In January only numerous scolices were present, and by February several worms had elongated somewhat but they were still immature. From June to November there were 20 to 30 mature worms present, and very few immature ones, but in December no mature tapeworms were found. Many of the scolices recovered in winter gave indications that they had once been strobilate. This parasite probably also requires insect intermediate hosts.

Five specimens of the acanthocephalan, *macracanthorhynchus ingens,* were recovered from the intestine of one of the skunks.

SUMMARY

1. Of the 14 skunks autopsied, each harbored an average of ten parasitic species and more than 100 individuals.
2. The commonest parasite was *Physaloptera maxillaris.* As many as 200 mature worms were recovered from the stomach of one skunk.
3. Only mature specimens of *Skrjabingylus chitwoodorum* were recovered.
4. Despite limited data, it appeared that mature specimens of *Physaloptera maxillaris* and *Oochoristica* sp. occurred in greatest numbers in June whereas immature forms occurred in greatest numbers in winter; that gravid *Arthrocephalus lotoris* were present in June but not in winter; that *Molineus patens* generally occurred in greater numbers in June than in winter; that *Isospora* sp. oocysts were being eliminated in greatest numbers in June; and that *Neotrichodectes mephitidis* were usually present in greatest numbers in winter. No seasonal trend was noted for any of the other parasites.
5. The skunk is a host of *Trichinella spiralis, Gongylonema pulchrum,* and *Dermacentor variabilis,* which are also parasites of man and/or livestock.
6. The genera *Gongylonema, Dipetalonema* and *Capillaria* have not previously been reported from skunks.

7. Other nematodes recovered were *Ascaris columnaris* and *Strongyloides* sp. Microfilariae were present in the blood of at least two hosts.
8. The trematode, *Alaria taxideae,* occurred in 3 of 14 animals. *Macracanthorhynchus ingens* was recovered once.
9. The tick, *Ixodes cookei,* occurred occasionally and a flea, *Ceratophyllus* sp., was recovered once.

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Eurytrema procyonis in a Raccoon from Connecticut

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An obviously ill raccoon, *Procyon lotor,* was picked up by a professional raccoon trapper near Washington Depot in Western Connecticut on March 19, 1953. He kept it alive until March 25, during which time it ate some of the food which was offered to it. On the 25th, it screamed suddenly, went into a coma, and died shortly thereafter. It was then brought to the University of Connecticut by Mr. L. A. Williamson of the State Board of Fisheries and Game for autopsy. Although five different species of helminth parasites were recovered from this animal, one of them, *Eurytrema procyonis* [=*Concinnum procyonis* (Denton, 1942) Travassos, 1944] is of particular interest in that it has been reported previously only from raccoons in Southeastern Texas. Stunkard and Goss (1950) considered that *Eurytrema vulpis provis.* Stunkard, 1947, is a synonym of this species. If so, this worm has also been recorded from the red fox, *Vulpes fulva,* in Rockland County, New York.

Denton (1942) first found and described this worm from the pancreas of 6 out of 10 raccoons collected near Lufkin, Angelina County, Texas, as *Eurytrema procyonis,* and later (1944) reported the common garden snail, *Mesodon thyroidus* (Say) as an experimental intermediate host. Denton also reported that there were from 12 to more than 1000 specimens in each infected raccoon and that the parasites lived in the inter-lobular ducts of the pancreas, with heavily infected raccoons showing no discernible effects from their infections. Travassos (1944) placed Denton's species in the genus *Concinnum* Bhalerao, 1936, several species of which have been reported from canine, feline, primate, and other mammalian hosts. Stunkard (1947) considered the validity of this doubtful and suggested the continued use of *Eurytrema* for this species.

In the autopsy of our Connecticut raccoon, the larger size and somewhat yellowish-pink color of the pancreas attracted attention as being different from the pancreases of numerous other raccoons that have been examined in our laboratories. It was soon discovered that the inter- and intralobular ducts were teeming with flukes identified as indicated above. By actual count, 2407 individual worms were found in approximately three-fourths of the pan-

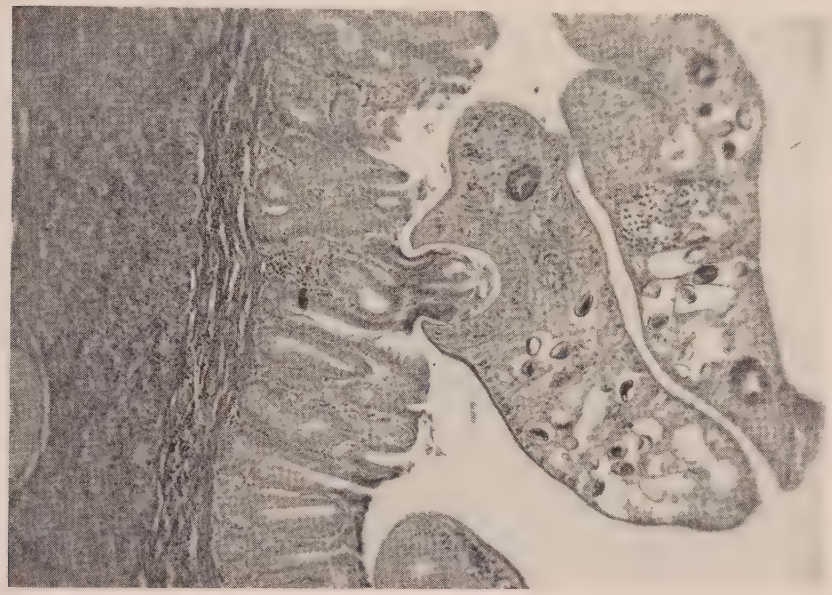

Figure 1. Photomicrograph of epithelial lining of duct of pancreas within grasp of acetabulum of *Eurytrema procyonis*.

creas, with the other fourth set aside for pathologic study. If this portion contained as many worms as the other, then well over 3000 worms were present.

Although the ducts were well packed with worms, sections of the pancreas did not reveal any major pathologic changes except that intralobular ducts containing worms (Figure 1) were greatly dilated and the epithelial linings of the ducts were often found to be within the grasp of the parasites' suckers. In connection with this it should be noted that the cloudy yellow urine had a pH of 5.8 by Nitrazine paper and that it was acetone, albumen, and sugar positive. In view of these findings it is believed that these parasites may have contributed to the illness and death of this animal.

It is probably not surprising to find unrecorded parasites of this sort in Connecticut. Known records of liberations of raccoons in the State were kindly supplied by Mr. L. A. Williamson of the State Board of Fisheries and Game, showing that 590 raccoons from at least seven states were released by game clubs from 1946 through 1952. These did not, so far as is known, include liberations of raccoons from any states south of Pennsylvania.

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An Abnormality of *Oncholaimus marinus* (Nematoda: Oncholaiminae)

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A single female specimen of *Oncholaimus* collected from marine algae on a breakwater at Woods Hole, Massachusetts, shows the presence of three distinct and fully-developed lateral tubes of the demanian system, opening behind the anus (fig. 1). (The terminology is that of de Man; the "lateral tubes" of de Man correspond to the "Endschlauch des Rohrorgans" of zur Strassen and are similar to the "moniliform glands" of Cobb.) The pores are lateral in position, two on the right side and one on the left. In morphological features other than the demanian system the specimen corresponds almost exactly to *Oncholaimus marinus* Schulz, 1932, as described more fully by Kreis (1934). A simple demanian system is reported for this species by both Schulz and Kreis, consisting of a rosette ("uvette" of Cobb, "Warze" of de Man) and an efferentus vaginalis. In those oncholaims which have the more complicated type of demanian system there are only two lateral tubes or moniliform glands, usually opening preanally (postanally in *Oncholaimium oxyure* var. *domesticum*).

Judging from the scarcity of references in the literature and from personal experience in observing hundreds of marine nematodes, abnormalities in these creatures seem to be rarely observed. This may be the first report of an abnormal duplicated structure in the marine nematodes.

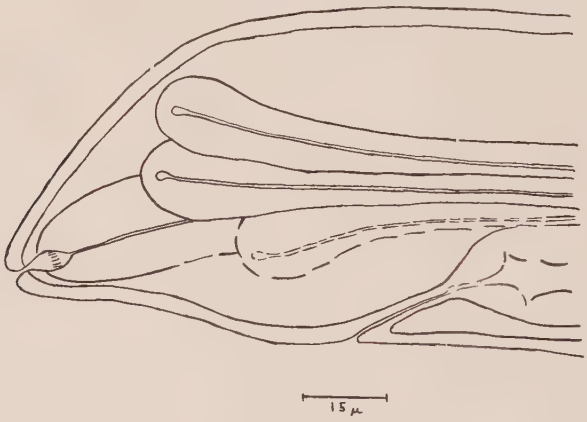

Fig. 1. *Oncholaimus marinus*, female tail showing pores of three lateral tubes.

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Haemoproteus Infections in Waterfowl

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Haemoproteus, an erythrocytic parasite first reported by Danilewsky (1889), is widely distributed in birds (Coatney, 1936; Herman, 1944). It is closely related to avian *Plasmodium* and *Leucocytozoon* and probably should be classified in the same family with these two genera. Levine and Hanson (1953) have reviewed most of the known records of *Haemoproteus* from waterfowl.

Laboratory transmission of *Haemoproteus* by means of hippoboscid flies has been accomplished in pigeons (Sergent, Ed. and Et., 1906; Aragão, 1908, 1916; Gonder, 1915) and mourning doves (Huff, 1932). O'Roke (1930) implicated hippoboscid flies as the vector of *Haemoproteus* in California quail. There have been no authentic reports of hippoboscid flies occurring on any Anatidae (Bequaert, 1953). Extensive studies of *Plasmodium* have been made possible by its transmissibility through blood transfusion. However, propagation of laboratory infections of *Haemoproteus* cannot be accomplished in this way since, as in *Leucocytozoon,* only sexual stages occur in the peripheral blood. This limitation has been a handicap in studying the biology of *Haemoproteus.*

The first report of *Haemoproteus* from Anatidae was by Johnston and Cleland (1909) from Australian teal (*Anas* (=*Nettion*) *castaneum*). They described *Halteridium nettionis* (later referred to by Cleland and Johnston, 1910, as *H. nettii*) as having typical *Haemoproteus* orientation in the host cell with microgametocytes taking a pale stain and macrogametocytes staining darker, with large pigment granules having a tendency to group at the ends of the elongated gametocytes. Furthermore, they reported that the nucleus of the host cell is displaced by mature forms to the point where it lies along one side of the parasitized cell with the gametocyte occupying all the cytoplasm.

Herman (1938) described *Haemoproteus* sp. from the common black duck (*Anas rubripes tristis*) from Cape Cod. The description of this parasite agrees closely with that of Johnston and Cleland.

On the basis of reported morphology, there appears to be no reason to differentiate most of the *Haemoproteus* which have been reported from Anatidae. The only observed consistent difference between most of the forms described from waterfowl is in the number of pigment granules in the gametocytes, as many as 30 having been reported. Kowarski, et al (1937) did not record this information but on the basis of their illustrations Levine and Hanson concluded that as many as 60 were present in two of their figured specimens. However, this method of interpretation may not be accurate. In the related genus *Plasmodium* some species, such as *P. vaughani,* have constancy in number of pigment granules whereas in *P. relictum* the number may vary from 8 to 32 in different strains.

Haiba (1948) reported *Haemoproteus* from the black duck (*Anas rubripes*) in Egypt with morphology similar to that described from the black duck by Herman (1938) and named the organism *H. hermani.* Herman (1951) reported further infections from ducks in California and used the name *H. hermani* for these forms. Since then, it has come to my attention that in an earlier paper Haiba (1946) referred to the same parasite as *H.*

anatis. His designations in 1946 and 1948 were based on the description given by Herman (1938) and presented no further diagnostic data.

Levine and Hanson (1953) have considered *H. hermani* to be a synonym of *H. anatis.* There can be no question about this synonomy even though Haiba's 1948 paper which named *H. hermani* contained no reference to his 1946 paper which designated *H. anatis.* Levine and Hanson designated their form from the Canada goose as *H. anatis.* Levine and Hanson further state "if future cross-transmission studies confirm the opinion that they all belong to the same species the correct name * * * would be *Haemoproteus nettionis,* (Johnston and Cleland, 1909) Coatney, 1936." The author recognizes no difference between his *H.* sp. and *H. nettionis* and thus recognizes the species of *Haemoproteus* described from North American waterfowl as *nettionis.* This status should be maintained unless or until cross-transmission experiments should prove otherwise. Accordingly, the following designations would be in order:

Haemoproteus nettionis (Johnston and Cleland, 1909), Coatney, 1936.
Type host: Australian teal, *Anas* (=*Nettion*) *castaneum.*
Synonyms:

Halteridium nettionis, Johnston and Cleland, 1909 from *Anas* (=*Nettion*) *castaneum.*

Halteridium nettii, Cleland and Johnston, 1910, from *Anas* (=*Nettion*) *castaneum.*

Haemoproteus sp., Rodhain, et al, 1913, from *Sarcidiornis melanota.*

Haemoproteus sp., Herman, 1938, from *Anas rubripes.*

Haemoproteus sp., Nelson and Gashwiler, 1941, from *Aix sponsa, Anas rubripes,* and *Aythya* (=*Nyroca*) *collaris.*

Haemoproteus sp., Kowarski, et al., 1937, from *Anas acuta, Asacornis scutulata, Aythya* (=*Nyroca*) *nyroca,* and *Netta rufina.*

Haemoproteus sp.,* Wetmore, 1941, from *Cygnus columbianus, Branta c. canadensis,* and *Anas p. platyrhynchos.*

Haemoproteus sp., Wood and Herman, 1943, from *Anas acuta tzitzihoa,* and *Anas p. platyrhynchos.*

Haemoproteus anatis, Haiba, 1946, from *Anas rubripes.*

Haemoproteus hermani, Haiba, 1948, from *Anas rubripes.*

Haemoproteus sp.,* Chernin and Sadun, 1949, from *Anas platyrhynchos.*

Haemoproteus hermani, Herman, 1951, from *Anas carolinensis, Anas platyrhynchos,* and *Spatula clypeata.*

Haemoproteus anatis, Levine and Hanson, 1953, from *Branta canadensis interior.*

Haemoproteus undetermined or questionably classified.

*Haemoproteus danilewskyi,*** Plimmer, 1912, from *Casarca* (=*Tadorna*) *tadornoides* and *Aythya* (=*Fuligula*) *baeri.*

*Haemoproteus danilewskyi,*** Plimmer, 1915, from *Cheniscus* (=*Nettapus*) *coromandelianus.*

Haemoproteus sp., Leger, 1918, from *Cairina* (=*Anas*) *moschata.*

Haemoproteus sp., Schwetz, 1931, from *Anas* sp.

Haemoproteus sp., Green *et al,* 1938 from *Anas platyrhynchus.*

Haemoproteus sp., Huff, 1942, from *Anas platyrhynchos.*

*The author has had opportunity to examine these specimens and believes they belong to this species.

**The author is in agreement with Levine and Hanson in the belief that this name is not acceptable for parasites of Anseriformes without proof of transmissibility. Valid *H. danilewskyi* is morphologically different from *H. nettionis.*

AMENDED DESCRIPTION OF *Haemoproteus nettionis*

Only the sexual stages occur in red blood cells of the peripheral circulation. Except during initial course of infection, young stages are absent or rare. Mature gametocytes usually displace the nucleus of the host erythrocyte and occupy all available cytoplasm. Frequently there is a narrow band of cytoplasm between the parasite and host cell nucleus. Occasionally, free gametocytes occur, usually in a rounded form. Pigment granules, usually present, vary from a few to as many as 30 or more, most frequently between 12 and 24. They may occur throughout the parasite but definitely show a tendency to orient at the poles. Pigment granules are usually coarse and rounded, although occasionally rod-shaped forms are observed. The host cell is not enlarged.

The microgametocytes stain pale bluish-pink with Giemsa's stain. The red-stained nucleus, though mostly concentrated in the central portion of the parasite, tends to diffuse throughout the organism. Male forms appear to be generally broader than female gametocytes and therefore to displace the host cell nucleus to a greater degree.

In the macrogametocytes, the cytoplasm is of alveolar structure, and stains blue with Giemsa's stain. Its nucleus, irregularly rectangular, is usually concentrated in the central portion of the parasite and stains red.

EXPERIMENTAL RESULTS

During the spring of 1952, blood smears were made from several incubating wood ducks (*Aix sponsa*) taken from nest boxes at the Patuxent Research Refuge, Laurel, Maryland. Three sampled on April 19 showed parasitemia with *Haemoproteus nettionis*. Four blood smears obtained similarly from wood ducks on May 1-3 demonstrated these parasites in only two of the birds. Negative findings resulted from examination of 29 approximately one-month-old wood ducks raised in captivity after hatching in an incubator.

Nelson and Gashwiler (1941), in their report of *Haemoproteus* from wood ducks, found that 49 of 77 birds (64%) were infected. Most of these birds were examined in the fall. In 53 juveniles, parasites were found in 44 (83%) while of 18 adults examined only in 6 (33%) was *Haemoproteus* evident. Four birds one to two weeks old were negative while two birds four to five weeks of age showed parasites. It has been pointed out (O'Roke, 1934) that the related genus *Leucocytozoon* produces a marked parasitemia in ducks during its initial course, but the parasites later disappear from the cells in the peripheral circulation or are present in very low numbers. In the spring, the parasites increase in numbers in the peripheral blood of carrier birds. At the same time there is an increase in abundance of insect vectors, thus enhancing the chances of transmission to new broods of ducks. Chernin (1952b) found that increases in numbers of *Leucocytozoon* parasites in peripheral blood invariably occurs in conjunction with onset of reproductive activity and egg laying. The high incidence of *Haemoproteus* infection in seven incubating wood ducks previously referred to may be an indication that the same relationship holds true for this parasite.

To determine if natural transmission of *Haemoproteus nettionis* was possible locally, 12 Indian runner ducks (*Anas platyrhynchos*) two weeks old and incubator-hatched at the Patuxent Research Refuge, were exposed to possible natural infection on June 27. Two cages made of ½-inch mesh hardware cloth, each containing six birds, were placed on the shore of two small ponds so that half the bottom was below water and half on land. At the end of one

week three birds from each cage were transferred to screened quarters, and the other six birds were moved at the end of two weeks. Blood smears were procured tri-weekly for 60 days. Two of the birds exposed two weeks showed *Haemoproteus* parasitemia July 30, 33 days after beginning and 19 days after end of exposure. The duration of parasitemia was 7 and 12 days, respectively.

As part of another study a number of white Chinese geese (presumed to be derived from *Cygnopis cygnoides*) was obtained at one day of age. These were kept indoors in brooders for several weeks and then placed in large concrete-floored runways about 100 yards from the shore of the pond where the Indian runner ducks had been exposed. One group of 56 geese was placed in the pens on May 20. Blood smears were procured fortnightly, and each smear was subjected to microscopic examination. Blood from three of these birds showed *Haemoproteus* on July 18, and in each of the next three examinations one additional bird developed parasitemia, for a total of six birds of the 56 (10.7%). One infected bird yielded only a single smear with parasites but parasitemia was found in two consecutive smears in the other five. In the latter the duration of parasitemia was more than two but less than six weeks. In a second group of 51 geese first exposed on June 25 when the birds were three weeks old, parasitemia was observed in three individuals (5.9%) on August 1, August 28, and September 13, respectively. In each of these, parasites were observed in two consecutive smears, indicating a duration of parasitemia of more than two but less than six weeks. All smears exhibited few parasites, rarely more than one per microscopic field. This is believed to be the first published report of *Haemoproteus* in any kind of domestic goose.

The morphology of *Haemoproteus* in Indian runner ducks and white Chinese geese was the same as that observed in wood ducks. The organism recovered therefore is diagnosed as *H. nettionis*. The vector of this parasite is apparently present at the Patuxent Research Refuge during the spring and early summer and probably is a free-flying insect since transmission occurred away from water as well as in a partially submerged cage. No ectoparasites were found on any of the birds in these experiments. The incubation period in domestic ducks was between 19 and 33 days. The first indication of parasites in a domestic goose occurred 23 days after initial exposure. The duration of parasitemia was shorter in ducks (7-12 days) than in geese (more than 2 weeks) and parasite density was greater in the ducks.

Chernin (1952a) has reported seasonal variation of *Leucocytozoon simondi* in domestic ducks in Michigan. In a survey of several scattered farm flocks in Emmet and Cheboygan Counties between June 23 and 30, 1948, blood smears of 42 ducks were all negative. Between July 10 and August 7, 37 out of 38 were positive. In 1949, between June 30 and July 5, of 42 ducks examined approximately half were infected while parasites were found in all 38 ducklings sampled between July 25 and July 28. Between June 30 and July 4, 1950, only about 15 per cent of 34 ducks examined showed parasitemia and almost all of 29 checked on August 8 were infected. He found that among 6-week-old ducklings exposed to natural infection for 8-day periods in late June or early July not all birds became infected and of those that developed parasitemia none died. In three successive test groups of birds exposed between July 11 and August 4, attack rates ranged from 90 to 100 per cent and fatalities from 14 to 83 per cent. Of 10 birds exposed in mid-August none developed parasitemia.

Findings on *Haemoproteus* at the Patuxent Research Refuge are essentially in accord with Chernin's findings on *Leucocytozoon* in ducks in Michi-

gan. Transmission of *Haemoproteus nettionis* occurred from early July through early August and source of infection was available to vectors as early as mid-April. Although the studies are limited, the results obtained with geese exposed from mid-May might indicate most active transmission during late June and early July and only limited transmission earlier or later.

SUMMARY

It is proposed that *Haemoproteus nettionis* (Johnston and Cleland, 1909) Coatney, 1936 be accepted as the correct name for the *Haemoproteus* of Anatidae. A list of synonyms and amended description of the parasite is given. Infections are reported from wood ducks (*Aix sponsa*) and from domestic ducks and geese, the last representing a new host record. Natural transmission was demonstrated at the Patuxent Research Refuge, Laurel, Maryland. Possible seasonal variation is suggested with active carriers present as early as mid-April among adult wood ducks and most active transmission occurring in June and early July with limited transmission earlier or later.

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Heterodera tabacum new species, A Parasite of Solanaceous Plants in Connecticut

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A *Heterodera* morphologically resembling *Heterodera rostochiensis* Wollenweber, the golden nematode of potatoes, was found in 1951 parasitizing tobacco on a farm at Hazardville, Connecticut (6). The occurrence on tobacco suggested that this nematode was not identical with the potato parasite. In tests made in England (5, 14), Germany (11), Holland (10) and the United States (9), *Heterodera rostochiensis* did not mature on tobacco.

Further tests of both the tobacco cyst nematode and the golden nematode showed that the two are distinctly different in their host preferences. The tobacco cyst nematode does not mature on potato (7, 12), preferred host of the golden nematode, but does mature on *Solanum nigrum* L., on all varieties of tobacco tested, and on *Nicotiana rustica* L. (7). The golden nematode has not been found to mature on any tobacco variety (2, 8), including those

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The authors wish to thank Mr. Gerald Thorne for guidance in this description. *Heterodera rostochiensis* material for comparison was kindly supplied by the Dept. of Plant Pathology, Cornell Lniversity; The Division of Nematology, B.P.I.S.A.E., U.S.D.A., Beltsville, Md., and Salt Lake City, Utah; the Division of Entomology and Parasitology, University of California, Berkeley; and the Nematology Dept., Rothamsted Experiment Station, England.

tested with the tobacco cyst nematode, or on *Nicotiana rustica* (2, 8), or on *Solanum nigrum* (4). The two nematodes have common hosts in *Solanum dulcamara* L., *Solanum integrifolium* Poir., *Solanum rostratum* Dunal, and *Lycopersicon esculentum* Mill (7, 12), but differences in degree of susceptibility of these hosts to the two nematodes are apparent.

In addition to these differences in host preferences a study of the tobacco cyst nematode and the golden nematode has revealed morphologic differences. These differences persist when the two nematodes are reared on the common hosts, *Solanum dulcamara, Solanum integrifolium,* and Marglobe tomato. The tobacco cyst nematode is therefore described and proposed as a new species.

Heterodera tabacum new species

MALE.[1] L = 710-1355μ (m = 1123μ, n = 134); a = 27-40 (m = 33, n = 30); b = 6.3-8.7 (m = 7.4, n = 6); c = 284-5325 (m = 1478, n = 29); T = 65-69% (m = 67%, n = 10). Spear Length = 24-27μ (m = 26μ, n = 24). Body slender, vermiform. Cuticle marked by transverse striae averaging 2 microns apart near middle of body. Subcuticle marked by duplex striations. Lateral fields ranging from $\frac{1}{3}$ body width at the median bulb to $\frac{1}{5}$ body width near the tail, beginning as 3 incisures about 15 microns from anterior end and increasing to 4 incisures just posterior to base of spear, extending around the tail. Outer incisures crenate. Head hemispherical with seven annules, the anterior one forming the labial disc. Amphid apertures minute, located beneath the border of the labial disc. First neck annule smaller than succeeding annules and placed under the head contour, setting off the head. Cephalic framework heavily sclerotized. Spear robust, with well developed knobs. Dorsal esophageal gland orifice 2.0-5.5μ (m = 3.6μ, n = 214) posterior to spear. Median bulb of esophagus ellipsoidal with well developed valve. The two subventral esophageal glands and the dorsal esophageal gland very variable in length and size, but commonly appearing as in figure 1A. In some specimens the dorsal esophageal gland is greatly reduced. Nerve ring large, encircling esophagus just anterior to junction with intestine. Excretory pore 10-13% of the body length from the anterior, opposite subventral glands. Testis $\frac{1}{8}$ body width at anterior end, increasing to $\frac{2}{3}$ body width toward posterior. Just adjacent to cap cell testis contains a double row of spermatocytes, and there are about 6 rows of spermatozoa anterior to the spicules. Spicules slightly arcuate, tips finely rounded, unnotched, 26 to 34 microns in length (measured along curve). Gubernaculum 9-12 microns long. Tail very short, 0.2-3.6μ (m = 1.9μ, n = 69),[2] lacking posterior protuberance (Fig. 1C). Phasmid located near terminus.

FEMALE. L = 327-688μ (m = 464, n = 50). Width = 206-516μ (m = 310, n = 50). Spear length = 18.5-24.1μ (m = 22.4, n = 10). Body ovate to spherical with elongate neck. Color white, becoming yellow as eggs mature. An outer layer of the cuticle marked by a rugose pattern (Fig. 3). An inner layer of cuticle marked by minute, often obscure punctations (Fig. 3). In the middle of the body these are usually arranged in rows running latitudinally. Between the vulva and the anus they either show no alignment or are aligned parallel to the vulvar-anal axis. Internal head sclerotization weak. Lip region variable, without well defined limits, apparently having 3 prominent annules,

[1]L = length; a = length divided by greatest width; b = length divided by esophageal length; c = length divided by tail length; T = length of testis expressed as percentage of body length; m = mean; n = number measured.

[2]Measured in lateral view.

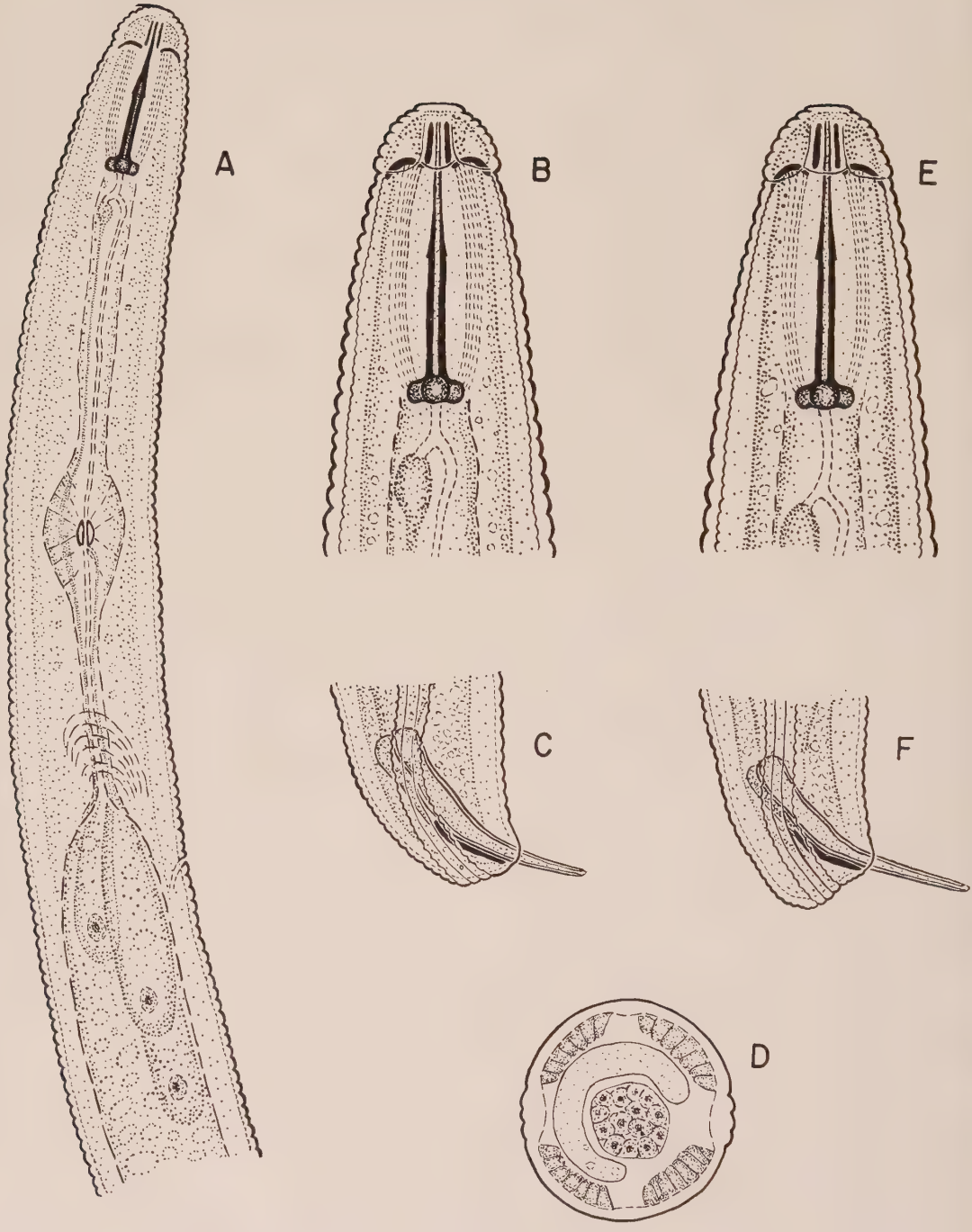

Fig. 1. *Heterodera tabacum* and *Heterodera rostochiensis* males. A. *H. tabacum* head and esophageal region (750×). B. *H. tabacum* head (1500×). C. *H. tabacum* tail (1000×). D. *H. tabacum* cross section through testis (1000×). E. *H. rostochiensis* head (1500×). F. *H. rostochiensis* tail (1000×).

and a labial disc (Fig. 2D). Spear with well developed basal knobs, and almost as stout as in male (Fig. 2D). Orifice of dorsal esophageal gland 3.8-8.0μ posterior to spear (m = 5.5μ, n = 20). Median bulb large and nearly spherical. Esophageal glands appear to be contained in a single lobe. Two ovaries fill the body cavity at maturity. Eggs 92-115μ x 43-50μ (n = 20). No markings observed on egg shell. Vulva not protruding from the body, situated ventrally from anus. Vulva slit-like, much larger than the anal opening.

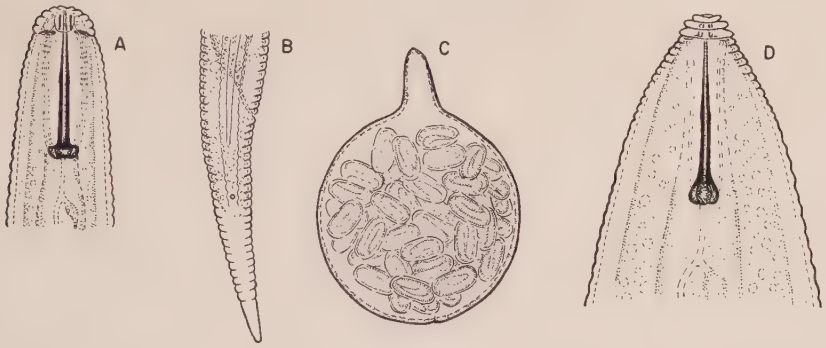

Fig. 2. *Heterodera tabacum*. A. Second stage larval head (1000×). B. Second stage larval tail (667×). C. Cyst (67×). D. Female head (1000×).

CYST (figs. 2C, 3A, and 3B). L = 337-740μ (m = 536μ, n = 131). Width = 232-645μ (m = 459μ, n = 131). Regression coefficient cyst length (including neck)/cyst width = 0.678 ± 0.035. Regression coefficient cyst width/cyst length = 1.101 ± 0.056. Shape, reticular pattern, and punctation as described for female. Cyst color—brown.

SECOND STAGE LARVA. L = 410-527μ (m = 476μ, n = 40); a = 22-24 (m = 23, n = 20); b = 4.1-4.9 (m = 4.4, n = 10); c = 8-11 (m = 10, n = 20); spear length = 22-26μ (m = 24, n = 20). Cuticle marked by transverse striae averaging 1.8μ apart at middle of body. Lateral fields cut by four incisures, extending from head to end of body cavity and slightly beyond phasmid (Fig. 2B), approximately one-fifth of body width at middle of body. Head (Fig. 2A) set off by a slight constriction and marked by four striae. Cephalic framework heavily sclerotized. Spear robust, with well developed basal knobs. Dorsal esophageal gland orifice 4.3-6.8μ (m = 5.5μ, n = 20) posterior to spear. Median bulb of esophagus ellipsoidal. Esophageal glands arranged as in male. Excretory pore 21-24% of body length from the anterior. Genital primordium 53-75% of body length from anterior. Phasmid about one-third distance from anus to tip of tail. Hyaline posterior portion of tail 22-31μ (m = 28μ, n = 40). Tail length 46-59μ (m = 52μ, n = 20). Terminus of tail finely rounded.

TYPE SPECIMENS. Holotype—male, Catalogue No. 31; Allotype—female, Catalogue No. 32; University of California Collection, Berkeley, Calif. Paratypes—The Connecticut Agricultural Experiment Station, New Haven, Conn.; The Division of Nematology Collection, B.P.I.S.A.E., U.S.D.A., Salt Lake City, Utah.

TYPE HOST. Tobacco, *Nicotiana tabacum* L.

TYPE LOCALITY. Hazardville, Connecticut.

HOST PLANTS. *Nicotiana tabacum* L., *Nicotiana rustica* L., *Solanum nigrum*

L., *Solanum dulcamara* L., *Solanum integrifolium* Poir., *Solanum rostratum* Dunal, *Lycopersicon esculentum* Mill.

Diagnosis. *Heterodera* with cyst rounded posteriorly, as in *Heterodera rostochiensis* Wollenweber, 1923 (15), *Heterodera punctata* Thorne, 1928 (13) and *Heterodera leptonepia* Cobb and Taylor, 1953 (1). *Heterodera tabacum* most closely resembles *H. rostochiensis,* but differs from this species in the following respects:

1. Male tail of *H. tabacum* shorter, 0.2-3.6 microns (m = 1.9μ, n = 69), than that of *H. rostochiensis,* 4.1-7.8 microns (m = 5.6μ n = 103) and lacks posterior protuberance (Fig. 1, A and F).
2. Distance between base of spear and dorsal esophageal gland orifice of male *H. tabacum* shorter, 2.0-5.5 microns (m = 3.6μ with standard error 0.06μ, n = 214) than in male *H. rostochiensis,* 5.1-10.9 microns (m = 7.4μ with standard error 0.13μ), n = 121).[3] (Fig. 1, B and E).

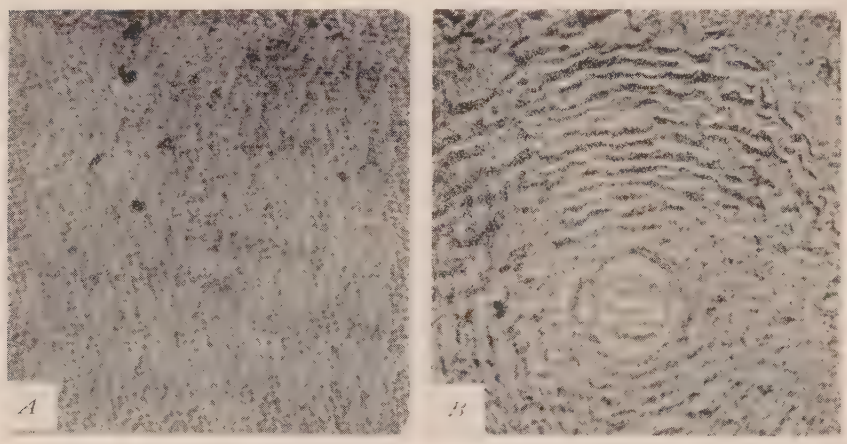

Fig. 3. *Heterodera tabacum.* Cuticular pattern of adult female. A. At center of body. B. In perineal region.

3. Head of male *H. tabacum* usually set off by a greater constriction than head of *H. rostochiensis* male . (Fig. 1, B and E).
4. Lip region of female variable in both species, but commonly with three prominent annules in *H. tabacum,* only two in *H. rostochiensis.*
5. Cuticular punctations between anus and vulva of *H. tabacum* commonly indistinct; when visible aligned parallel to vulvar-anal axis, or showing no alignment. Punctations between vulva and anus of *H. rostochiensis* usually perpendicular to vulvar-anal axis.
6. *H. tabacum* matures on *Solanum nigrum L.* and on tobacco, but not on potato. *H. rostochiensis* matures on potatoes, but not on *S. nigrum* and tobacco.

H. tavacum can be separated from *H. punctata* by cyst shape (spherical in *H. tabacum,* oblong in *H. punctata*), and by the relative size of the vulvar and anal openings. These appear to be about equal in *H. punctata* since the anus is located on a thin spot on the cyst wall (3). Vulvar opening

[3] A single aberrant measurement of 2.8μ excluded.

of *H. tabacum* cysts is much larger than anal opening. *H. tabacum* can be readily distinguished from *H. leptonepia* on the basis of second stage larval characters. Mean larval length of *H. tabacum* is 476 microns as compared to 567 microns for *H. leptonepia*. The larval dorsal esophageal gland orifice averages 5.5 microns behind spear in *H. tabacum*, about 12 microns behind spear in *H. leptonepia*.

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Neorhabditis, a New Name for *Pararhabditis* Schuurmans Stekhoven

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Dr. E. W. Price of the Zoological Division, U. S. Bureau of Animal Industry, has called my attention to the fact that *Pararhabditis* Schuurmans Stekhoven 1951 is preoccupied by *Pararhabditis* Baylis and Daubney 1926. Consequently, the new name *Neorhabditis* is proposed for this homonym.

The Tapeworm Genus *Wyominia* Scott, 1941

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Neuchâtel

In the form of an abstract, J. W. Scott (1941) gave a short account of a new genus and species of tapeworm collected from the bile ducts of the Rocky Mountain bighorn sheep *Ovis c. canadensis* Shaw, from Wyoming. He named the parasite *Wyominia tetoni* n. gen., n. sp., and announced that a more complete description with figures would appear later. The latter has, however, never been published and in a personal letter Dr. Scott informs me that he has handed over all of his material to the Zoological Division of the Bureau of Animal Industry in Beltsville, Maryland. I am very grateful to Dr. E. W. Price for allowing me to examine several fragments of this worm and to study its anatomy from sections.

The total length is not recorded. However, a series of five specimens measured 70, 80, 100, 150, and 200 mm, averaging 120 mm, with a maximum breadth of 6 mm. The scolex bears four pedunculated suckers each measuring 1.1 to 1.2 mm in diameter. The greatest width of the scolex, measured at the level of the suckers, is 2.3 mm. All the segments are broader than long and the posterior border of each segment is slightly lobed. Unfortunately, the material is not very well preserved and a number of anatomical details must be omitted pending further investigations. The longitudinal musculature is rather weakly developed and tends to form two layers of irregular bundles consisting of fairly large fibers. Dorso-ventral muscle fibres appear to be very numerous. The thick-walled excretory vessels are very conspicuous, both the ventral and the dorsal vessels having approximately the same diameter

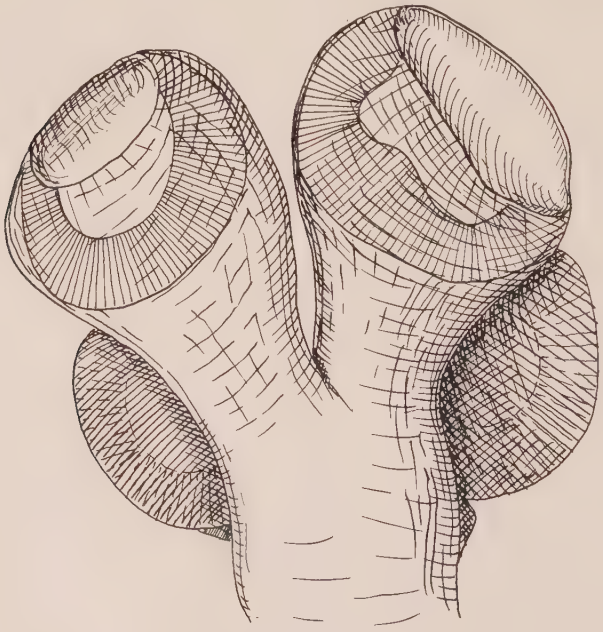

Fig. 1. Scolex.

of 28 to 30 μ. The dorsal vessels are situated medially to the ventral vessels, the latter being united in each segment by a transverse vessel. Secondary vessels, arising from the main stems, do not appear to be present. There are two sets of genitalia but, apparently, a single uterus. The genital ducts pass between the longitudinal excretory vessels and dorsal to the nerve cord. The cirrus pouch opens into a male genital atrium located on the lateral border of the segment and capable of becoming everted. The cirrus pouch is rather long and narrow, very often somewhat coiled, its proximal end reaching to the ventral excretory vessel. It measures 206 μ to 230 μ in length and 32 μ to 27 μ in diameter. The unarmed cirrus is very narrow and the vas deferens is coiled within the proximal portion of the pouch. Before entering the latter, the vas deferens forms a dense coil surrounded by deeply staining unicellular glands that lies between the dorsal and ventral excretory vessels. There are from 75 to 80 large testes each of which is 78 μ to 90 μ in diameter. This number of testes is almost twice as great as that stated in the original description, but since the latter was undoubtedly based upon a whole mount this would account for the discrepancy. Moreover, the testes do not occur in a single row in the anterior third of the segment but are found, two rows deep, almost throughout the entire medulla, limited on either side by the ovaries. The vagina does not lead into the male genital atrium but opens directly onto the dorsal surface, on one side, and onto the ventral surface on the other, at some distance from the cirrus pouch (figs. 2 and 3). It forms a large receptaculum seminis situated between the ovary and the yolk gland. Owing to the poor state of preservation, it is difficult to discover the exact limits of the latter glands. The uterus first appears as a transverse tubular organ situated in the *anterior* third of the segment and more or less surrounded by the testes (fig. 4). The eggs, at first very small, fill the uterus but I have not been able to discover any paruterine organs in the fragments available. Mr. Allen McIntosh has, however, sent me a single gravid segment from a bighorn sheep collected in the Kofa Refuge, Arizona, in which there are numerous paruterine organs that appear to be fibrous capsules. The latter are more or less spherical to oval, measuring 160 μ to 200 μ by 91 μ to 114 μ, and containing each about six thin shelled eggs. These are 45 μ in diameter and contain an embryo 22 μ by 17 μ. The larval hooks are 10 μ long.

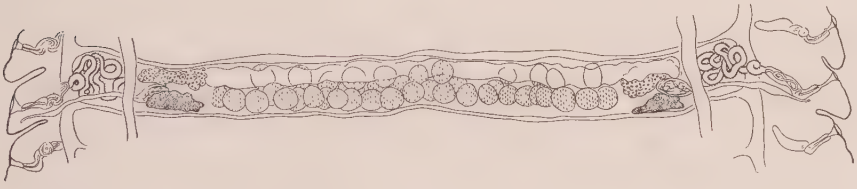

Fig. 2. Partly reconstructed horizontal section passing through an adult segment.

It is clear from the above description that the original diagnosis of *Wyominia tetoni* does not conform with the anatomy as revealed by sections. Unfortunately, the material at hand has not made it possible to study the origin and further development of the paruterine organs the structure of which is very reminiscent of the fibrous capsules occurring in gravid segments of other members of the sub-family *Thysanosominae* Fuhrm.

Fig. 3. Portion of a transverse section showing the relative position of the male pore and vagina. Cp—cirrus pouch; Ga—everted male genital atrium; Vag—vagina opening onto the dorsal surface of segment; Vd—vas deferens surrounded by "prostatic" cells.

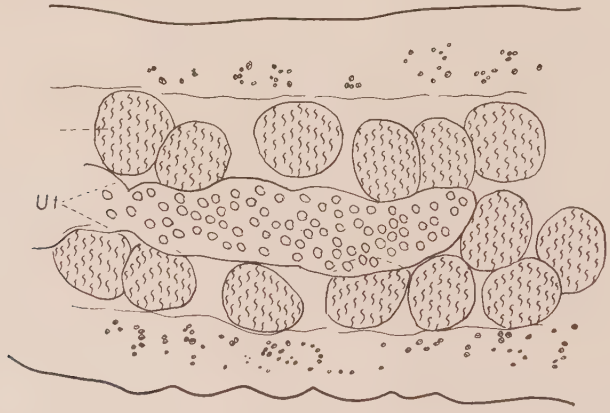

Fig. 4. Portion of a transverse section showing the relative position of the testes and uterus. T—testes; Ut—uterus.

Wyominia bears a rather close relationship to *Thysanosoma*, another monotypical genus that also occurs in the bile-ducts of ruminants from both North and South America.* It differs, however, from the latter by the position of the vagina, the structure of the uterus and the presence of a yolk gland. It is really most regrettable that no detailed description of the ultimate development of the paruterine organs of *Thysanosoma* has ever been published

*Wardle & Macleod (1952, p. 379) refer to *Th. actinioides* as "a cosmopolitan parasite in stock raising countries." A statement that is incorrect since all the reports of this worm outside of the New World are found to be erroneous, or due to confusion with the genus *Helictometra*.

and also that the origin of these organs in *Wyominia* have not yet been described. When such data become available, it will be possible to obtain a clear insight into the possible relationships of these two genera.

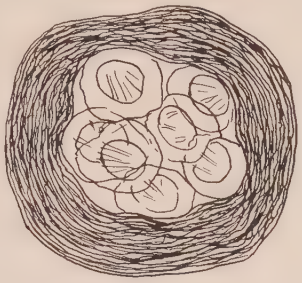

Fig. 5. Isolated paruterine organ containing eggs.

In view of the present study it will be necessary to redefine the genus from the bighorn sheep as follows:

Wyominia Scott, 1941—

Scolex with pedunculated suckers. Posterior border of segments more or less lobed. Two sets of genitalia per segment. Genital ducts pass between the longitudinal excretory vessels and dorsal of the nerve cord. Male genital atrium lateral. Vaginal opening at a certain distance from the male atrium onto the dorsal surface of the segment on one side and onto the ventral surface on the opposite side. Testes large, distributed throughout the entire segment, limited laterally by the female glands. Yolk gland present. Uterus a large, transverse tube in anterior half of medulla. Gravid segments containing numerous, ovoid, paruterine capsules enclosing about six eggs each.

Adult in the bile-ducts and duodenum of *Ovis c. canadensis* Shaw.

TYPE SPECIES: *Wyominia tetoni* Scott, 1941.

The subfamily *Thysanosominae* contains the following seven genera: *Anootypus* Woodland, *Ascotaenia* Baer, *Avitellina* Gough, *Helictometra* Baer, *Stilesia* Railliet, *Thysanosoma* Diesing, *Wyominia* Scott, all of which with the exception of the last one show various degrees of atrophy of the yolk gland together with a distinct reduction of the capacity of the uterus. These genera occur exclusively in ruminants and, curiously enough, only the last two of the above mentioned genera are found in wild ruminants from the New World, whereas all the others occur in Old World ruminants exclusively. This original distribution pattern may, however, be changed by introduction of parasitized sheep and cattle from the Old World into North and South America. The presence of *H. giardi* reported by Antequeda (1936) from sheep in the Argentine Republic may be explained in this way.

The relationships of the Old and New World ruminants is a moot point and their probable common origin has not yet been determined with any degree of accuracy. The presence of two distinct groups of tapeworms one in each part of the World, would tend to show that the two host groups have been isolated from one another for a great length of time. Since atrophy of the yolk gland is obviously a secondary character in tapeworms, the genus *Wyominia,* where this gland is retained, would be more primitive than *Thysanosoma* that no longer possesses such a gland. Both these genera are double-pored whereas all of the Old World genera are single pored. It is therefore

of great interest to find that the genus *Crossotaenia* Mahon (in press) from the bile-ducts of an African antelope, although single-pored shows certain affinities with *Wyominia* except that there are no paruterine organs and that the ova are very large with stippled shells. Consequently, this genus belongs to the *Anoplocephalinae,* but in view of certain morphological affinities with *Thysanosoma* and *Wyominia,* the question arises whether this is just a question of convergence or an example of phylogenic relationships?

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Occurrence of the Lungworm *Protostrongylus boughtoni* Goble and Dougherty, 1943 in Snowshoe Hares (*Lepus americanus bairdii*) in Colorado

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During the course of an ecological study of snowshoe hares from Crystal Mountain, at an elevation of about 8,500 feet, approximately 18 miles west of Fort Collins, Colorado, four were examined postmortem for parasites.* All were infected with the lungworm *Protostrongylus boughtoni* Goble and Dougherty, 1943 (J. Parasitol. 29: 397-404).

Examination of our material showed general agreement in morphological details with the description and figures of *P. boughtoni* by Goble and Dougherty. One variation occurred, however, in the shape of the mouth. Whereas these authors represented it as being round in their figure 5, our material showed it as being triangular with the angles somewhat rounded.

While Scott (1943, Univ. Wyo. Publ. 10: 57-71) reported and described *P. sylvilagi* from cottontails (*Sylvilagus nuttalli grangeri* (Allen, 1895)) and whitetailed jackrabbits (*Lepus townsendi campanius* Hollister, 1915) in the vicinity of Laramie, Wyoming, he did not find *P. boughtoni.* Neither did we find *P. sylvilagi* in the snowshoe hares which came from an area not far from that of Scott's specimens.

The lungs of the snowshoe hares showed lesions similar to those described by Scott for cottontails from Wyoming and by Green and Shillinger (1935: Rep. Minn. Wildlife Disease Invest. Dec., pp. 91-95 (mimeogr.)) for varying hares from Minnesota. Whether these parasites were causing mortality among the hares in Colorado was not ascertained.

This report constitutes an extension of the geographic range of *P. boughtoni.*

*Appreciation is expressed to Ralph W. Meeks who collected the hares and brought them to the laboratory for examination.

Inheritance of Resistance to a Root-Knot Nematode (*Meloidogyne incognita* var. *acrita* Chitwood) in *Vitis* spp.*

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The root-knot nematodes, *Meloidogyne* spp., are serious, destructive pests in California vineyards. There are many localized areas of infestation in the sandy soils of central and southern California. In these regions the *Vitis vinifera* grape varieties grown on their own roots have repeatedly shown injury from root-knot nematode attack expressed by a weakening of growth and a marked decrease in productivity.

Nematode resistant grape rootstocks have been in commercial use in California vineyards for some years. The clone (*V. solonis* × Othello) 1613 has been a popular rootstock in many areas where nematode resistance is needed for establishing grape vines. Several other rootstocks, especially clones of *V. champini* Planch., have shown promise in experimental plantings and are now being used on a limited commercial scale in California vineyards. Fruiting varieties grafted to these stocks, however, have not been entirely satisfactory, either because of poorer fruit quality or lower yield.

The nematode resistant rootstocks presently available to commercial vineyardists have not met with their general approval. Since certain *Vitis* species were indicated as carrying a high degree of resistance, a study of the inheritance of root-knot resistance in various *Vitis* species should provide a basis for the breeding of new and more satisfactory grape rootstock varieties.

The work presented in this paper is divided into two parts. One is to investigate the possibility of variability existing within diverse collections of the root-knot nematode by testing rootings of different *Vitis* species to them. The other is to test progenies from controlled crosses between chosen species of *Vitis* to one collection of root-knot nematode and to present a hypothesis for the genetic constitution of the species concerned in regards to resistance to this pest.

Neal (1889) was the first to study the destructive effects of root-knot nematodes on perennial plants in this country. It is interesting to note that as early as 1889 he clearly recognized the importance of using immune varieties as rootstocks. His work is the first reported on resistance and susceptibility of *Vitis* species. He recognized the susceptibility of *Vitis vinifera* varieties and suggested the use of *V. cordifolia* and *V. vulpina* as resistant rootstocks. Snyder (1936) tested 154 *Vitis vinifera* grape varieties at Shafter, California, and found all to be severely infested. Further tests, however, indicated that several species of *Vitis,* of American origin, were quite resistant. *V. champini, V. solonis, V. doaniana,* and *V. cinerea* appeared to be the most promising as carrying inherent resistance to nematode injury.

The first comprehensive study of the inheritance of nematode resistance in a plant species was that of Nilsson-Ehle (1920). His tests on several varieties of barley, *Hordeum vulgare,* with the sugar-beet nematode, *Hetero-*

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I am indebted to Professor H. P. Olmo, Department of Viticulture, for originally suggesting this problem and his numerous helpful discussions during its pursuit. I also extend my thanks to Professor M. W. Allen, Department of Entomology and Parasitology, for his assistance in identification and classification of the nematode collections used in this study.

*dera schachtii,** indicated that immunity in those varieties is conditioned by a single dominant gene. Barrons (1940) hypothesized that in *Phaseolus vulgaris* there exists two dominant genes for susceptibility, the homozygous recessive individuals being resistant plants. McFarlane, Hartzler and Frazier (1946) conducted breeding tests on the inheritance of resistance to root-knot in tomatoes using the resistant species *Lycopersicon peruvianum* × susceptible *L. esculentum.* An analysis of the segregation obtained in the F_1 and F_2 generations indicated that *L. peruvianum* is heterozygous for a dominant gene for resistance. Watts (1947) working with the same cross, *L. peruvianum* × *L. esculentum,* found evidence from a selfed population arising from a backcross seedling that resistance in *L. peruvianum,* at least in the early stages of plant growth, is conditioned by two dominant factors.

Weinberger, Marth and Scott (1943) studied several foreign introductions of peaches for root-knot resistance. Seedlings of Yunnan and Shalil were both reported as resistant. In crosses with susceptible varieties only resistant plants were found. Yunnan and Shalil were reported as homozygous dominant for resistance. Havis, et.al., (1950) also tested peach seedlings for resistance. They introduced the idea of species within the recent classification of nematodes in the genus *Meloidogyne* and found that Yunnan (P.I. 55886), though quite resistant to *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, was susceptible to the species *M. javanica* (Treub, 1885) Chitwood, 1949. The Lovell variety was susceptible to both species and a new rootstock variety S-37 (Plant Pat. No. 904) was found resistant to both.

In presenting the classification of the root-knot nematodes within the genus *Meloidogyne* Goeldi 1887, Chitwood (1949) has indicated that morphologically several species can be recognized.

The work of Christie and Albin (1944), Christie (1946) and Christie and Havis (1948) raised the question of races being formed and maintained as a result of host-parasite relationships. Allen (1952) has shown that several populations of one species of root-knot nematode collected from cotton in California displayed a wide diversity of host plant specificity. Studies of this nature have brought to light not only the variability within the plant material but also that within the populations of plant parasitic nematodes themselves. In the light of such results it would appear necessary to re-examine much of the past work on the inheritance of resistance to root-knot, to evaluate the question of racial and specific differences which are now evident among the diverse populations of root-knot nematodes.

Experimental Methods

Eighteen samples of vineyard soils infested with root-knot nematodes were collected in March, 1948. Cultures of nematodes from these soils were established on tomato plants of the variety San Marzano. A Yolo fine sandy loam soil was steam sterilized at 15 pounds pressure for three hours, placed in 12-inch pots and a series inoculated with equal portions of chopped roots from each of these collections. These series of infested soils were used to test the resistance of rootings of various *Vitis* species. Specimens from these 18 collections were identified as all belonging to the same species, *Meloidogyne incognita* var. *acrita* Chitwood, 1949.

Seedling tests were conducted in three metal tanks on benches in the greenhouse. The tanks were 3 feet by 8 feet and deep enough to hold 8 inches

*The species of nematodes which he tested against is probably *Heterodera major* (Schmidt, 1930) Franklin, 1940.

of infested soil. The soil was inoculated with a root-knot nematode collected at Davis, the original inoculum being taken from infected carrot plants. This collection, identified as *Meloidogyne incognita* var. *acrita,* was cultured on tomato plants, the roots chopped, and an equal amount of inoculum added to each tank. Bottom heat was supplied to each tank through 60 feet of soil cable rated at 400 watts. The temperature of the soil mass fluctuated between 24° C and 27° C throughout the tests. Overhead lighting from 6:00 p.m. to 10:00 p.m. was used during the winter months for the first four weeks of seedling growth in the tanks. One application of approximately 40 gms. of ammonium sulfate fertilizer, dissolved in water, was sprinkled over each tank ten days after the seedlings were placed in the infected tanks.

The seedlings were grown from grape seeds germinated in sterile soil in greenhouse flats having removable bottoms. When the seedlings had developed two true leaves, the flat was placed in position in the tank of infested soil and the bottom removed. This technique facilitated the transfer of large numbers of seedlings without disturbing their original position in the flats. Ten rows of 25 seeds each were planted in each flat, with eight flats being accommodated by each tank at one time. Each seed lot was replicated four or more times at random through the tanks. Approximately one-fifth of all the seedlings tested at one time were selfed *V. vinifera* varieties and can be considered susceptible checks.

The time that the seedlings remained in the tanks was varied to some extent in order to attain an equal amount of growth on each lot of seedlings tested. The cooler night temperature and less intense light in the greenhouse during winter months reduced the growth rate for the seedlings. During such periods the seedlings were allowed to stay in the tanks a few days longer than the lots run during the summer. The period of time that the different lots remained in the tanks varied from 45 to 60 days.

At the end of the period of infection the seedlings were removed from the tanks. The roots were washed clean of soil with running water and each seedling examined for infection using a binocular microscope with a magnification of 18X. Finally five cuttings each of all the plants used as parents in the crosses were rooted in the tanks and their root systems rated for infection.

All plants that were examined were placed in four classes of infection, 0 = free from infection, 1 = slight infection, 2 = moderate infection, and 3 = severe infection. In the data for the seedling tests the three infected classes are grouped together and all the plants classified into one of two categories, infected or clean.

EXPERIMENTAL RESULTS

Table I shows the results of testing rooted plants of several *Vitis* species to the 18 field collections of the root-knot nematode. Three cuttings of each species were rooted in each test, with the exception of *V. rotundifolia.* Since it is almost impossible to cause cuttings of varieties of this species to root, seedling taken from the variety Scuppernong were used in this test instead of rootings.

All the rootings at the time of examination were classified using the ratings discussed above. The dash (—) indicates that the cutting failed to root or rooted so poorly that no classification for infection could be made.

In the samples numbered 11 and 15, where a reversal of reaction was noted, two supplementary 12-inch pots of each inoculum were set up and

TABLE I.—Resistance Ratings of Some Vitis Species to 18 Field Collections of Root-Knot Nematode

Coll. no.	Source*	*vinifera* 3 var.	*solonis* 3627	*berlandieri* 3602	*champini* 3639	*labrusca* 3632	*rotundifolia* seedlings	*rupestris* St. George
1	Weedpatch	3	0	0	0	3	0	1
2	Arvin	3	0	–	0	–	0	2
3	Arvin	3	0	0	–	3	0	3
4	Arvin	3	0	–	0	–	0	2
5	Arvin	3	0	2	–	3	0	2
6	Delano	3	0	–	–	3	0	1
7	Delano	3	0	1	0	–	0	3
8	Delano	3	0	–	–	–	0	–
9	Exeter	3	0	0	0	3	0	1
10	Dinuba	3	0	–	0	–	0	2
11	Dinuba	3	3	3	3	3	0	3
12	Kingsburg	3	0	–	0	3	0	2
13	Fowler	3	0	3	–	3	0	3
14	Madera	3	0	2	–	3	0	3
15	Madera	3	3	3	3	3	0	3
16	Lodi	3	0	–	0	3	0	2
17	Red Bluff	3	0	–	0	3	0	3
18	Santa Rosa	3	0	–	0	–	0	2
19	Check (uninfected	0	0	0	0	0	0	9

*All locations are in California.

rootings of *V. solonis, V. champini* and *V. berlandieri* and seedlings of *V. rotundifolia* were planted in two successive tests. In each case the results obtained in the original test were confirmed.

Results of the seedling tests on population of plants involving susceptible *Vitis* species is presented in Table II. The seedling tests on crosses within the species *V. vinifera* and *V. labrusca* and the test on populations arising from selfed varieties within these species show that they both transmit a very high degree of susceptibility to their offspring. This information tends to confirm Snyder's (1936) conclusions on the susceptibility of *vinifera* varieties. The assumption that the two species are completely susceptible and will transmit this susceptibility to their offspring seems to be in order.

The *V. champini* clone 3639, showed a high degree of resistance when rootings were tested in infested soil. However, its reaction in the crosses shown in Table III indicates that it cannot be considered as transmitting complete resistance to all its offspring. The *champini* × *vinifera* seedlings segregated very close to a 1:1 ratio, suggesting a single gene difference between the two speices, with *champini* being in the heterozygous condition.

TABLE II.—Seedling Tests on Populations Arising from Susceptible *Vitis* species

Cross	Type of Cross	No. of Plants infected	No. of Plants clean	Ratio of clean/infected
Molinera (*vinif.*) selfed	sus. × sus.	31	0	—
Carignane (*vinif.*) selfed	sus. × sus.	39	0	—
Pierce (*vinif.* × *lab.*) *selfed*	sus. × sus.	97	0	—
Eaton (*vinif.* × *lab.*) selfed	sus. × sus.	17	0	—
Hunisa (*vinif.*) × Scolokertek (*vinif.*)	sus. × sus.	93	4*	—
V. labrusca × Scolokertek (*vinif.*)	sus. × sus.	24	0	—
V. labrusca × Champion (*lab.* × ?)	sus. × sus.	37	0	—
Hunisa (*vinif.*) × J17:54**	sus. × res.	5	55	11.0:1
Hunisa (*vinif.*) × J17:64	sus. × res.	18	38	2.1:1
Hunisa (*vinif.*) × J17:14	sus. × res.	22	47	2.1:1
Hunisa (*vinif.*) × J17:47	sus. × ?	20	42	2.1:1
Almeria (*vinif.*) × J17:12	sus. × res.	23	42	1.8:1
Hunisa (*vinif.*) × J17:16	sus. × sus.	61	29	0.5:1

*These plants were very weak, likely escapes.
**All J17 plants are F_1 *V. champini* × *V. rupestris* made available from the rootstock breeding project of the Department of Viticulture.

The *rupestris* clone 3620, behaved as a susceptible when rootings were tested. The fact that resistant plants arise from a population derived from *vinifera* × *rupestris* crosses indicates, however, that the rupestris plant must also carry genetic factors for resistance. On this basis the genes contributing the resistance to some of the *champini* × *vinifera* hybrids cannot be acting the same as those which appear in the resistant plants present in the *rupestris* × *vinifera* hybrids.

Table III also presents the results of the test on some backeross populations of individual plants from a F_1 population of *V. champini* × *V. rupestris*. The reaction of these F_1 plants in the rooting tests is indicated under the column entitled "type of cross." Certain trends can be noted from this data which substantiates that shown in the first part of Table III. The susceptible F_1 plants when crossed to *champini* or *vinifera* gave ratio comparable to that found when the parent V. *rupestris* was crossed to these plants. The populations obtained using resistant F_1 plants, however, gave ratios indicating that they carried as much or more resistance than their resistant *V. champini* parent. This information upholds the hypothesis that the *rupestris* clone, 3620, must also carry genetic factors for resistance.

During the summer of 1951 seed lots were collected from open pollinated plants of several *Vitis* species in their natural habitat in Arizona and central Texas. The tests on plants from these seeds along with some populations of seedlings from interspecific crosses are shown in Table IV. In these tests *V. candicans* reacts as a resistant species. The few infected plants from population 51101 were consistently different in leaf type and vigor from the typical *candicans* seedlings. They could possibly have been the result of cross pollination in the field of the *V. candicans* female with another species of grape occurring adjacent to it.

TABLE III.—Seedling Tests on Populations Arising from *V. rupestris* and *V. champini*

	Type of Cross	No. of Plants infected	clean	Ratio of clean/infected
Hunisa (*vinif.*) × *V. rupestris* (3620)	sus. × sus.	53	21*	0.4:1
V. labrusca × *V. rupestris* (3620)	sus. × sus.	63	13	0.2:1
V. champini (3639) × Scolokertek (*vinif.*)	res. × sus.	42	41	1.0:1
V. champini (3639) × Thompson seedless (*vinif.*)	res. × sus.	15	19	1.3:1
V. champini (3639) × Champion (*lab.* × ?)	res. × sus.	24	53	2.2:1
V. champini (3639) × *V. rupestris* (B 123:18)	res. × sus.	22	66	3.0:1
V. champini (3639) × *V. rupestris* (3620)	res. × sus.	35	107	3.3:1
J17-44 × *V. rupestris* (38131)	res. × sus.	32	84	2.8:1
J17-45 × *V. rupestris*	res. × sus.	10	28	2.7:1
J17- 9 × *V. rupestris*	res. × sus.	21	56	2.6:1
J17-63 × *V. rupestris*	sus. × sus.	56	48	0.9:1
J17-59 × *V. rupestris*	sus. × sus.	25	26	1.0:1
V. champini (3639) × J17-12	res. × res.	19	118	6.2:1
V. champini × J17-54	res. × res.	3	71	23.6:1
V. champini × J17-13	res. × res.	15	167	11.1:1
V. champini × J17-64	res. × res.	27	168	6.2:1
V. champini × J17-69	res. × res.	9	82	9.1:1
V. champini × J17-16	res. × sus.	51	168	3.3:1
V. champini × J17-56	res. × sus.	12	14	1.2:1
V. champini × J17-61	res. × sus.	15	72	4.8:1
V. champini × J17-65	res. × sus.	14	24	1.7:1
V. champini × J17-47	res. × ?	17	62	3.6:1

*One replication high in resistant plants.

TABLE IV.—Tests on Open Pollinated Species Seedlings and Certain Other Crosses

	Type of Cross	No. of plants infected	clean
V. candicans (5169)	—	0	59
V. candicans (51101)	—	9*	25
*V. monticola*** (51115)	—	23	25
V. monticola (51119)	—	26	2
V. monticola (51120)	—	42	28
V. Treleasei (5162)	—	89	59
V. rotundifolia (Scuppernong)	—	0	119
V. solonis × A × R #1 (*vinif.* × *rup.*)	res. × sus.	64	36
(*V. solonis* × Othello) 1613 × A × R #1	res. × sus.	47	49
(*V. solonis* × Othello) 1613 × *V. candicans*	res. × res.	0	27
V. champini (3639) × *V. candicans*	res. × res.	2	103

*Susceptibles in this cross were all off-type plants.
**This species appears closely allied to *V. rupestris*.

The *V. monticola* collections, which appear to be closely allied to the species *V. rupestris*, showed a wide variability in the segregations of their populations. The figures shown would indicate that the samples of this species which were collected carry genes both for resistance and susceptibility, which conforms with the reaction obtained in the seedling tests with the *V. rupestris* plants.

Both the species *V. solonis* and the rootstock (*V. solonis* × Othello) 1613 appeared as resistant plants in the cutting tests. When crossed to the susceptible A × R #1, both plants show that they do not transmit resistance to all the seedlings derived from the cross. 1613 when crossed with *V. candicans* gave all resistant plants. The resistance coming from *candicans* is therefore dominant over susceptibility of the 1613.

CONCLUSIONS

In order to evaluate the response of the various species of *Vitis* studied and the seedling populations of their hybrids to the root-knot nematode, it was necessary to evaluate the variability which exists among the diverse populations of the nematode itself. The tests conducted on the species rootings demonstrate that this variability does exist and can be detected by the difference in reaction of certain of the species to the different field collections of the nematode. This variation in reaction indicates that subspecific or racial differences exist among these nematode collections and is reflected in the behavior of some of the grape species tested.

Since this variation among the collections of nematodes is apparent, it was felt necessary to select a specific representation of the nematode species *Meloidogyne incognita* var. *acrita* and conduct all the seedling tests on this single race of root-knot nematodes. It is necessary to understand, therefore, that the results obtained in these seedling tests can be extended only to the single population of nematodes cultured for the study and cannot include the wide range of diverse types of root-knot nematodes which seem to exist in the warm, sandy agricultural areas of the state. On the other hand, a study of this type can yield information concerning the nature of the resistance which is evident in some species of *Vitis* and, with further testing, certain resistant lines or combinations of them might lead to the production of more desirable resistant grape rootstock varieties.

There are certain possible relationships among the American species of grapes which would be appropriate to discuss here. Planchon (1882) and later Viala and Ravaz (1903) have placed *V. champini* as a population of

plants of hybrid origin arising through intercrossing between *V. candicans* and *V. rupestris*. Both *V. candicans* and *V. rupestris* are widespread species in the wild state. They are well defined in their morphological characteristics throughout their range. The *V. champini* population, on the other hand, is much more limited in range, and seems to show a greater amount of inherent variability than either of the other two species.

The seed collections of *V. candicans* from Texas indicate that this species is homozygous resistant to root-knot nematodes whereas *V. champini* segregates both resistant and susceptible plants when crossed to *V. vinifera*. This supports the idea of hybrid origin, namely, that *V. champini* is *candicans* × *rupestris*.

On the basis of the facts discussed above we might then assign a tentative genetic constitution for the species concerned. Evidence is presented which indicates that *V. vinifera* and *V. labrusca* are both completely susceptible species and contain in common in the homozygous condition the same gene or genes for susceptibility. *V. candicans* appears, on the other hand, to be homozygous dominant for resistance. If the supposed hybrid derivation of *V. champini* is correct, then it should contain a dominant gene or genes from *V. candicans*. Since *V. champini* segregates 1:1 in crosses with *V. vinifera*, a single dominant gene coming from *V. candicans* is indicated.

SUMMARY

1. Within the genus *Vitis*, some species tested show a high degree of inherent resistance to the root-knot nematode, *Meloidogyne incognita* var. *acrita* Chitwood 1949.
2. The variability in reaction of *V. solonis* and *V. champini* to diverse collections of this nematode have indicated that within a single recognized variety of root-knot nematode there exist racial differences in ability to produce root-knots in species of *Vitis*.
3. Evidence is presented which indicates that *V. champini* is heterozygous and *V. candicans* homozygous for a dominant gene conferring resistance to root-knot nematode. Both *vinifera* and *labrusca* are homozygous recessive and hence susceptible.

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The Occurrence of *Physaloptera rara* Hall and Wigdor 1918 in Dogs in Pennsylvania

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Physaloptera rara was described as a new species of nematode from the dog in Michigan by Hall and Wigdor (1918). Since that time, according to Morgan (1944), it has been found in dogs from Wisconsin, Michigan and Tennessee. He also indicated that this parasite was recorded in 14 other carnivores and that its occurrence has been in no less than 14 states. Secord (1933) and Baker (1941) also reported immature *Physaloptera* from dogs in Ohio and New York, respectively.

In December 1948, the author necropsied two mongrel pups, two to three months of age, which came from a farm near Annville, Lebanon County, Pennsylvania. In one dog, upon opening the stomach, the presence of eight pinkish-white nematodes was noted. One of these worms was a male, attached to the mucosa. The other dog harbored two worms similar to those found in the first animal. A morphological study of these parasites definitely placed them in the genus Physaloptera.

The male worm measured 25.8 mm. in length and 741.8 μ in diameter. The oesophagus was 4.4 mm. long. The right spicule was 55.6 μ and the left 691.3 μ in length. The arrangement of the caudal papillae and phasmids conformed to those described by Ackert (1936) for *P. felidis* (=*P. rara*) and by Morgan (1944) for *P. rara*. The females, with the exception of one, were immature. The measurements and morphological characters of the mature female and the morphological features which could be determined in the immature worms, also conformed to those given for *P. rara*. This seems to be the first record of this parasite occurring in dogs in Pensylvania.

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The Domestic Sheep a New Host for *Cooperia bisonis*

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Cooperia bisonis was described in 1925 by Cram (J. Agric. Res. 30: 571-573) from material collected from buffalo at the National Buffalo Park, Wainwright, Alberta, Canada. Additional reports of the occurrence of this nematode seem to be limited to the United States. Morgan and Hawkins (1949, Vet. Helminthol., Burgess Publishing Co.) mention that it was reported on two occasions from cattle in Wyoming and Montana. These reports probably refer to a publication by Price (1929, J. Parasitol. 15: 219-220) who recorded the occurrence of the nematode in Wyoming cattle and to some specimens from Montana cattle which are in the Helminthological collection of the U. S. National Museum. In 1945 Lucker and Dikmans (Proc. Helminthol. Wash. 12: 2-4) reported its occurrence in the pronghorn antelope. So far as the writers have been able to ascertain, *C. bisonis* has not previously been found in domestic sheep.

We recently found male and female nematodes in the small intestine of a lamb originating near the Eastern New Mexico-West Texas line which appear to be *C. bisonis**. Our specimens agree in general with Cram's description, with the exception of a relatively small difference in the length of the spicules. While Cram gives a length of 224 to 240 microns for the spicules, those in at least one of our specimens measures slightly longer, about 260 microns.

According to Cram, *Cooperia bisonis* is most closely related to *C. onchophora,* with principal differences pertaining to the presence or absence of a vulvular appendage and the position of the ventral branches of the dorsal rays. In *C. bisonis* the vulva is covered by a large projecting linguiform process and the ventral branches of the dorsal rays originate near the junction of the rays with the stem. In *C. onchophora,* on the other hand, there is no vulvular appendage and the ventral branches of the dorsal rays originate near a point midway between the junction of the rays with the stem and the distal ends of the rays.

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The Cattle Nodular Worm in Connecticut

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Infections of the cattle nodular worm, *Oesophagostomum radiatum* (Rudolphi, 1803), a parasite which is known to have deleterious effects on cattle, are being acquired by cattle born and reared in Connecticut.

Although interstate cattle harboring infections of the cattle nodular worm may have been slaughtered in Connecticut, this has not been generally recognized. To our knowledge it has not been reported from Connecticut or from any of the other New England states.

In the belief that oesophagostomes might be present in the University of Connecticut herds, we instigated routine checks at the time of slaughter of steers from these herds destined for the meat cutting classes. One steer killed on March 11, 1953, had a medium-light infection of typical nodules containing larvae in the small intestine and cecum. The second infected steer, killed March 23, 1953, had small numbers of adults in the colon as well as the nodules in the small intestine and cecum. The worms collected have been identified as larvae and adults of *Oesophagostomum radiatum*.

The nodules which we observed were fairly typical of early infections reported by Andrews and Maldonado (1942). They were raised areas 1 mm or more in diameter and a few of the nodules were showing hemorrhage in the second steer examined. Some of the nodules were filled with pus in both animals.

The infected steers came from the herd kept at the University of Connecticut. The first steer killed was an Angus born May 13, 1951, on pasture back of the University beef and sheep barn. It was wintered there during 1951-52, fed hay and grain, and pastured near there during the summer of 1952. It was put on a grass silage experiment in November. The second steer was a Hereford born June 23, 1951, on pasture at the Spring Hill Farm. It was wintered in the beef and sheep barn on hay and grain, pastured at the Spring Hill Farm pasture with beef cows during the next summer and put on a corn silage experiment in November of 1952. All food used came from University of Connecticut fields.

Andrews and Maldonado (1941) found that in Puerto Rico the eggs of *Oesophagostomum radiatum* hatch in about 2 days to become 3rd stage infective larvae 5 or 6 days later. Due to the climatic differences, the rate of preparasitic development may be longer in Connecticut than in Puerto Rico. The local problem is to be given further study.

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